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THE ABSORPTION OF ELECTROLYTES IN LARGE PLANT CELLS

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INTRODUCTION

As this subject is most advantageously studied in large multinucleate cells the present review will be largely confined to such investigations.

The protoplasm of these cells forms a thin layer (not over 10 microns thick) outside of which is a cellulose wall and inside which is a large central vacuole filled with sap. At the inner and outer surfaces of the protoplasm are non-aqueous layers between which is an aqueous phase.

The advantages of such cells are manifold. Injury is easily detected (especial attention has been devoted to avoiding it in our experiments). The sap can be obtained with little or no contamination in sufficient quantities for analysis, without recourse to microchemical methods. Substances can be introduced into the cell without permanent injury by means of a capillary piercing the wall. In *Halicystis* two such capillaries were inserted by Blinks and left permanently in the cell so that the internal vacuole could be irrigated; *e.g.*, its sap was replaced by that of *Valonia* (of very different composition) and the cell continued to live (3). Another great

advantage is that an electric current can be sent through the capillary so as to pass only once through the living protoplasm.

In studying these cells new problems and points of view have been encountered. When we learned what was going on inside the cell we had to get rid of many preconceptions. For example, we soon found that the higher concentration of potassium inside the cell is not due to the formation of insoluble compounds (99) or to the Donnan equilibrium (p. 294).

Some puzzling facts have turned up which, perhaps, must wait for their interpretation until physical chemistry has made further progress, especially in relation to non-aqueous solvents. Certain principles, however, have become increasingly clear and some of these will be briefly discussed.

The main themes are: the kinetics of penetration, accumulation, selective permeability, and the nature of the protoplasmic surface.

THE KINETICS OF PENETRATION

This appears to be conditioned by the fact that the protoplasm has at the surface a non-aqueous layer, immiscible with water, through which entering electrolytes must pass. This is shown by a variety of evidence (99, *967*), including electrical measurements on these and other cells.[1] An illustration of the latter is seen in the work of Fricke and Curtis (37) which shows that the surface of the yeast cell is composed of a non-aqueous layer of little or no conductivity. Rapidly growing yeast may take up a large quantity of electrolyte which must pass through this layer in the form of undissociated molecules, or of undissociated neutral complexes which carry no current and which, consequently, from our present point of view, are like molecules. For convenience we may, therefore, in the present discussion call them molecules. This may be illustrated by considering the penetration of acids.

Acids. It follows from what has been said that the rate of movement of a weak acid H*A* across the non-aqueous surface layer of the protoplasm will be proportional to the concentration gradient of its molecules in that layer. At the start of penetration we may neglect the internal concentration and may consider merely the concentration of molecules in the outer surface of this layer. This

[1] For work on these cells see 3, 4, 5, 6; bibliography in 99. For work on cell suspensions see 34, 35, 36, 37, 38; 24, 25, 26, 27.

will be proportional to the product (H) (A) in the external solution (under the simplifying assumption that there is no dissociation in the non-aqueous layer and that concentrations are equal to activities in all cases).

This is confirmed by the experiments of A. G. Jacques (75) on the penetration of H_2S into *Valonia* (see also 9, 69, 76).

In the case of weak acids it is usually assumed in the literature that the undissociated molecules penetrate more rapidly. This is to be expected on the basis of what has just been stated; for a fuller discussion see p. 303.

It is possible that this treatment can also be applied when HA is a strong acid, for if H^+ and A^- collide at the surface they may form a molecule which can pass into the non-aqueous layer just as when HCl passes from water into air. On this basis the rate of entrance would be proportional to the number of collisions[2] and hence to the product (H) (A). It follows that in the case of a strong acid (with no dissociation in the non-aqueous layer) multiplying H^+ and A^- by two would multiply the product (H) (A) and, consequently, the rate of entrance by four.[3] This could not happen in the absence of a non-aqueous layer. This conception has not received an adequate test but there is evidence that strong acids enter more rapidly at low pH (45, 46, 48, 127). Regarding the entrance of HNO_3 see 126.

Since the rate of entrance appears to depend chiefly on the two non-aqueous layers (99, *1013*)[4] which may, for convenience, be treated as a single layer (84), we may assume that the rate of entrance of acids will be proportional to $(H_o)(A_o) - (H_i)(A_i)$

[2] We arrive at the same result by assuming, for purposes of calculation, that a few molecules of HA exist in the external solution.

[3] This would cause the partition coefficient to increase with concentration. This has been observed in the case of models (97, *400*; 116, 117) but varies in different compounds. It is affected by the degree of dissociation in the non-aqueous phase. In the case of KCl distributed between water and guaiacol the increase in the partition coefficient is much less than in the case of potassium guaiacolate.

[4] An analogous situation is found in certain models (p. 297) in which the protoplasm is represented by a layer of guaiacol which is vigorously stirred but which has unstirred layers at each of its surfaces. The unstirred layers correspond to the non-aqueous surface layers of the protoplasm and the intermediate stirred portion corresponds to the aqueous layer of the protoplasm where the transport of many substances (*e.g.*, salts) is presumably much more rapid than in the non-aqueous layers; this is apparently due to their low partition coefficients (p. 301) rather than to diffusion constants (*cf.* 84).

where the subscripts *o* and *i* refer to outside and inside. Since the chemical potential is proportional to the product (H) (*A*), the rate will depend on the difference in chemical potential, *i.e.*, on the driving force (when there is dissociation in the non-aqueous layer, this will be less exact and in any case it is only an approximation).[5]

Bases. In the case of bases the concentration gradients in the protoplasm appear to depend on chemical combination. As an illustration, we may consider the penetration of NH_3 into *Valonia* (100).[6]

When we add NH_4Cl to the sea water it soon makes its appearance in the sap, entering chiefly as NH_3[7] (or NH_4OH). If NH_3 penetrated by simple diffusion,[8] the rate of entrance would be proportional to $NH_{3o} - NH_{3i}$ (where the subscripts *o* and *i* refer to concentrations outside and inside, respectively). This is not the case. As $NH_{3o} - NH_{3i}$ increases, the rate fails to keep pace with it but appears to approach a limit (Fig. 1).

How can this be accounted for? A simple explanation, suggested by the study of models (p. 297; also 99, *992*) is that NH_4OH combines with a constituent HX of the protoplasm according to the equation $NH_4OH + HX \rightleftarrows NH_4X + H_2O$. Then NH_4X diffuses inward,[9] so that the rate of entrance depends on the concentration of NH_4X in the protoplasm rather than on that of NH_3 in the external solution.

The relative amount of NH_4X found at the outer surface of the protoplasm at each concentration of NH_{3o} may be ascertained from the formula

$$(NH_{3o})\ (HX_b - NH_4X_e) = k\ (NH_4X_e)$$

where k is a constant and the subscripts b and e refer to molar concentrations at the beginning and when the reaction has reached

[5] There are complicating factors (109) and, as explained later, the rate will be proportional also to the permeability of the protoplasm which depends on its chemical composition and on its structure.

[6] Regarding the penetration of weak electrolytes into these cells see, 13, 31, 54, 55, 57, 58, 63, 69.

[7] This is shown by adding .001 M NH_4Cl to sea water and changing the pH. As the pH is lowered the rate of entrance falls off very rapidly (100).

[8] Regarding laws of diffusion see 71, 122, 123, 125.

[9] NH_4X is presumably formed in the outer non-aqueous layer of the protoplasm and diffuses through this layer. It may not be very soluble in water and may pass through the aqueous layer of the protoplasm chiefly in some other form (*e.g.*, as NH_3 or as NH_4Z) but this will be left out of account in the present discussion.

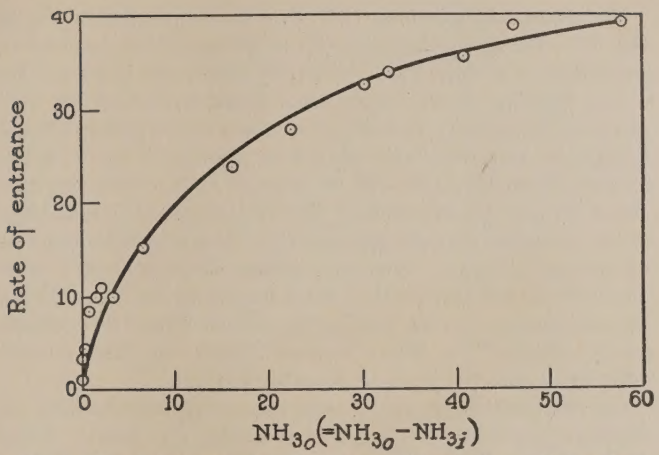

FIG. 1. Graph indicating that NH_3 enters *Valonia* by combining chemically with the protoplasm. The rates of entrance (moles of NH_3+NH_4 entering 1 liter of cells in 10 minutes) are plotted as ordinates and the values of $NH_{3o}=NH_{3o}-NH_{3i}$ as abscissae, where the subscripts o and i refer to concentrations outside and inside, respectively; circles show observed values. See 100.

It is assumed that NH_3 enters by combining with a constituent of the protoplasm, HX, to form NH_4X, and that the rate of entrance is directly proportional to the concentration gradient of NH_4X. The rate of entrance is taken as proportional to the concentration of NH_4X at the outer surface since in such brief experiments its concentration at the inner surface is negligible.

The curve shows the rate of entrance calculated on the basis of the reaction $NH_3+HX \rightleftarrows NH_4X$ from the formula $(NH_3)(HX_b-NH_4X_e)=.001533\ (NH_4X_e)$ where the subscripts b and e refer to the beginning of the reaction and to equilibrium, respectively (HX_b is taken as .005 M).

The value of NH_{3i} is taken as zero because the experiments last only 10 minutes and most of the NH_3 which enters during this time is changed to NH_4^+ on account of the low pH of the sap.

equilibrium, respectively. The curve in Fig. 1 is obtained by putting $k=.001533$ and $HX_b=.005$ M.

On reaching the sap, NH_4X may react with HCl to form NH_4Cl, or with CO_2 to form NH_4HCO_3 after which HCO_3^- may be exchanged for Cl^- coming in from the outside: we suppose that HCO_3^- and Cl^- move chiefly in molecular form, *i.e.*, as CO_2 and HCl, through the non-aqueous surface layer of the protoplasm since these layers, being immiscible with water, must have low dielectric constants which permit little dissociation (116).

The strong base guanidine (74) gives a curve resembling that in Fig. 1.[10] Perhaps this applies also to lithium whose entrance is, according to Collander (28*a*), relatively more rapid in dilute solutions. Although similar experiments cannot be carried out with potassium (because it penetrates very slowly and is already present in high concentration in the sap) there is evidence that it enters *Valonia* chiefly as KOH (99, *985*; also 77, 79). We find that unless the ionic activity product [K][OH] is greater outside than inside, potassium does not enter the cell, and it penetrates faster as the product increases. When this product becomes greater inside (owing to an increase in OH_i) potassium leaves the cell, although sodium continues to enter because the product [Na][OH] remains greater outside (77). This is shown in Fig. 2. In *Nitella* no such effect of external pH has been detected (80).

On this basis we should say that potassium enters because the chemical potential of KOH is greater outside than inside. If we assume that in the sap KX reacts with CO_2 to form $KHCO_3$ and that HCO_3^- is exchanged for Cl^- coming in from outside, we have a possible picture of the process (99, *983*). (We suppose that HCO_3^- and Cl^- move mostly in molecular form through the non-aqueous surface layer whose low dielectric constant permits little dissociation.)

If the exchange of HCO_3^- or other organic anions were not complete we should find cations in the cell paired with organic anions to a considerable extent. This has actually been demonstrated (72, 73) in flowering plants.

When bases enter as hydroxides we may expect certain relations among which are the following:

(1) Increased production of carbon dioxide (and other organic acids) will promote growth and the absorption of electrolytes:[11] the reasons for this are clearly seen in models (99, *1002*; 81, 83, 108). (It is commonly said that the energy needed for accumulation is due to respiration but in the model we can bring about accumulation without deriving any energy from the formation of carbon dioxide. We merely employ it after it has been produced

[10] If the partition coefficient increased rapidly enough with concentration[3] the first part of the curve might be convex to the horizontal axis. Our present data do not permit us to decide whether this occurs.

[11] *I.e.*, by favoring the entrance of bases and by providing anions for exchange with inorganic anions in the external solution.

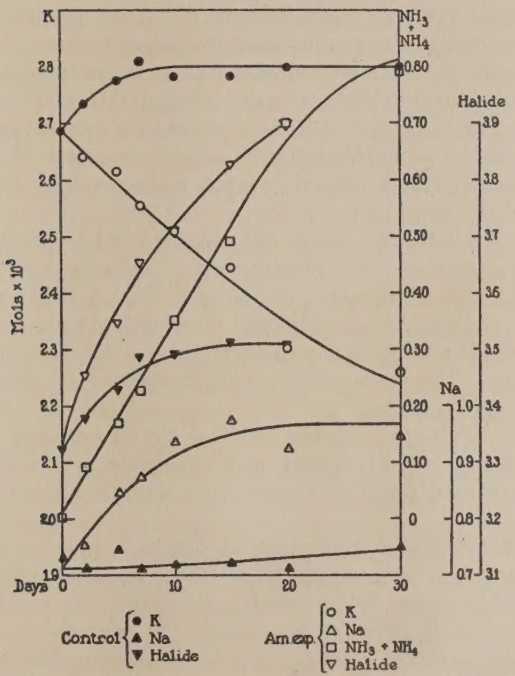

FIG. 2. Graphs showing the change in moles of K, Na, $NH_3 + NH_4$, and halide in a typical lot of cells of *Valonia macrophysa* in sea water (control) and in an equivalent lot of cells in sea water containing .001 M NH_4Cl (the scales of ordinates for each substance are the same but are displaced vertically to bring the curves into one figure). The entrance of NH_3 is accompanied by a rise in the pH value of the sap (not shown here) which causes K to come out while Na continues to go in. It should be noted that the ordinates do not refer to concentrations but to the total number of moles in a certain lot of growing cells. The growth is proportional to the increase in moles of Cl in the control (where the concentration of Cl remains nearly constant) and this is approximately true for the cells exposed to NH_4Cl (where a small increase in the concentration of Cl occurs). (77).

elsewhere, thus using what is usually regarded as a waste product of the cell.)

This is quite in harmony with the fact that, in general, the greatest growth and absorption of electrolytes are found when there is the greatest production of carbon dioxide, as in germinating seeds, in flower formation and at growing points generally (99, *984*; see also 42, 43, 86).

Steward (119, 120) and Steward and Berry (121) state that absorption of electrolytes increases as respiration increases but not necessarily in direct proportion. This is to be expected for if potassium enters as KOH, the value of $(K_o)(OH_o) - (K_i)(OH_i)$ will not necessarily be doubled by doubling the rate of respiration. They think that other kinds of vital activity also play a rôle. Other investigators find a relation between respiration and absorption (44, 86, 87, 114, 114*a*).

(2) Absorption of cations and growth would be favored by a rise in external pH within certain limits.[12] The limits will depend on the nature of the case. Beginning at the lowest pH at which the cell can grow, we should expect the growth to increase with increasing pH until secondary changes[13] occur which have an inhibiting effect. This seems to be generally the case (79). Hence there is an optimum pH.

Salts. Evidently a salt like NH_4Cl might enter as such or might combine to form NH_4X, but if such combination occurs it must be very restricted for NH_4Cl enters very slowly as compared with NH_3.[14]

A similar state of affairs appears to exist in certain models (p. 296), in which the protoplasmic surface is represented by the organic acid guaiacol (which may be called HG). Here NH_3 enters by forming NH_4G which on reaching the "artificial sap" reacts with CO_2 to form NH_4HCO_3; this applies also to potassium. We find that NH_3 and KOH enter rapidly[15] but NH_4Cl and KCl penetrate very slowly, as with *Valonia.* However, if KCl enters *Valonia* as such, it does so against a gradient; this applies also to NH_4Cl when its concentration is higher inside, but it enters very slowly (if at all) as NH_4Cl even when it goes with the gradient.

The anion of the salt may enter together with H^+ which will make the external solution more alkaline (126). See p. 303.

[12] This takes place when green cells are illuminated (99, *986*) and may explain the favorable effect of light on absorption in some cases.

[13] A possible example of such changes is seen in *Nitella* when exposure to alkaline solutions causes great changes in the properties of the protoplasmic surface (104).

[14] For the rapid penetration of NH_3 (*i.e.*, of NH_4X) as compared with NH_4Cl see 100. The more rapid penetration of KOH as compared with KCl is indicated by the fact that when the external pH rises, potassium enters more rapidly although the external concentration of KCl remains the same (79).

[15] Regarding acetic acid and postassium acetate see p. 303.

Rôle of ionic transfer. These experiments indicate that electrolytes pass through the non-aqueous protoplasmic surface chiefly in molecular form since its low dielectric constant would not permit much dissociation. That some dissociation takes place and that ions can enter to some extent is shown by the bioelectrical effects (99, *997*), but the concentration of ions is very small, as shown by the high electrical resistance and impedance of the surface (p. 284; also 99, *1012*; 24, 25, 26, 27, 35, 37, 38). Owing to the low dielectric constant of the protoplasmic surface, all electrolytes will be weak in the surface layer (116) and the transport of ions through the surface must play a very subordinate rôle (99, *994, 1004*).

This applies whether we suppose the ions to enter as dissociated molecules (anions and cations entering together) or by ionic exchange (*e.g.*, K^+ entering in exchange for H^+ coming out) as assumed by some investigators (50; 68; 99, *994*). For equations for the exchange of ions see 95.

With *Valonia* it is possible (by making certain assumptions) to calculate the rate of entrance by ionic exchange as compared with that on the basis described above and in every case the result is adverse to the idea of ionic exchange (99, *994*; 78).

If the entrance of electrolytes depended chiefly on ionic transport, it should be possible to predict quantitatively the relative rates of entrance from the apparent mobilities of the ions in the protoplasm, but this is not possible (99, *1006*).[16] For example, on the basis of ionic mobilities we should predict that cesium would enter about as rapidly as sodium,[17] but it penetrates very much more slowly (30).

In view of this, it is not surprising that our experiments yield evidence against the suggestion of Michaelis (99, *990*) and of Höber that the entrance of electrolytes is determined by the charge on the surface of the protoplasm. For example, in *Valonia* this concept meets with difficulties, since with KCl we should on this basis have to say that the surface is negatively charged (as the more dilute solution is electrically positive in the external circuit). But, as Damon has shown, with NaCl we should have to conclude

[16] Regarding the penetration of strong electrolytes see (14, 15, 17, 18, 19, 22, 30).

[17] Damon (unpublished results) finds that the apparent mobility of Cs^+ in *Valonia* does not differ greatly from that of Na^+.

that the surface has a positive charge, since the more dilute solution is negative (32).

This difficulty can be avoided by ignoring the effect of any possible charge on the surface. We can then calculate the apparent relative mobilities of K^+, Cl^- and Na^+ in the non-aqueous surface layer to be 20, 1 and .2, respectively. Damon finds (unpublished results) that the order of mobilities is Rb > Cl > Na; this is the order found in water and it seems reasonable for a non-aqueous layer. This would account for the electrical behavior on the basis of diffusion potential without any assumption regarding a charge on the surface.

If the surface charge determined the entrance of electrolytes it is difficult to see how both anions and cations could enter in equal numbers, as happens in *Valonia;* for a negative charge would inhibit the entrance of anions and a positive charge that of cations. There is no difficulty, however, when we ignore the effect of charge and suppose the protoplasmic surface to act like a non-aqueous layer, *e.g.,* guaiacol plus quinoline, through which K^+ and Cl^- pass in equal numbers (unpublished results).

The views here presented are at variance with those of several authors who maintain that electrolytes enter chiefly by ionic exchange. Regarding these, see 8, 8*a,* 8*b,* 15, 17, 21, 38*a,* 50, 51, 68, 90, 91.

Permeability of the protoplasm. The rate of entrance depends not only on concentration gradients in the protoplasm but also on the permeability of the protoplasm which may be defined for HA as the number of moles entering in unit time under standard conditions[18] when the value of $(H_o)(A_o) - (H_i)(A_i)$ is unity. This definition arises from the conception that the rate of entrance is proportional to $(H_o)(A_o) - (H_i)(A_i)$ and cannot be more than an approximation (p. 285). It applies to weak acids but not to Fig. 1 except where the curve approximates a straight line. It is, nevertheless, useful when its limitations are recognized.

Owing to variability[19] of the protoplasm, the safest method is to make all measurements comparative, using the same substance as a control in every case.

[18] *I.e.,* of temperature, area, etc. (*Cf.* 99, *990*).

[19] This depends on both its chemical composition (*cf.* 60) and its structure (p. 293). For methods of determining permeability see 99, *979;* 68, 69, 69*a,* 70, 70*a,* 71, 71*a,* 84*a,* 84*b.*

Nature of the time curve. Before concluding this section it is desirable to call attention to changes of concentration with time. In *Valonia* and *Nitella* the time curve for entrance of certain dyes (11, 53) and of bromide (49) is of the first order. This applies also to the exit of dyes (56). In some cases experiments indicate the second order for *Valonia* (10, 75, 76) but this might result from variability in the permeability of the cells. For if the time curve were really of the first order and in some cells (*e.g.,* in those of smaller size or greater permeability) penetration were completed more rapidly, the process would appear to proceed more rapidly at first and then slow down when the average of all the cells was taken (99, *998*).

In these brief experiments there is little or no entrance of water; for equations see 93, 95. When water enters, the situation changes and for equations dealing with this see 82, 83, 84, 98; also 70.

Temperature coefficient. The temperature coefficient is, in general, high. This does not necessarily indicate a chemical reaction though this seems to occur in certain cases (p. 286). For dyes, Irwin found $Q_{10} = 4$ or more (53, 56); Hoagland, Hibbard and Davis (49) found Q_{10} between 2 and 3 for the entrance of bromide. Irwin found $Q_{10} = 2.3$ in a chloroform model (66).[20]

Some very important aspects of kinetics can be more conveniently treated in later sections, *e.g.,* partition coefficients (p. 301); steady state (p. 296).

Influence of one substance on another. The diffusion constant and the partition coefficient of any substance in the non-aqueous layer may be affected by the presence of other substances.

A variety of factors may affect the rate of penetration (79, 81, 82, 83, 84, 107, 109, 110, 111) and when the entering electrolyte enters into chemical combination with the protoplasm (100), one substance may affect the entrance of another by competing for the substance which acts as a carrier (99, *999*) if the latter is limited in amount. An influence might be exercised (99, *999*) by any substance which alters the viscosity, thickness or chemical composition of the protoplasmic surface, as seen, for example, in antagonistic action or in the effects of distilled water (106) or in changes in the pH of the protoplasm or of its surroundings (99, *999;* 79).

Of especial interest are the other variables which have been dealt

[20] For recent theoretical considerations see 1, 33.

with by Irwin (54, 55), McCutcheon and Lucké (88), Hoagland, Davis and Hibbard (45, 47, 48). When the absorption of potassium changes as the external concentration of calcium increases (85), the result may depend on physiological balance which affects the state of the surface (*cf.* 99, *999*).

Very striking results were found by Irwin (60). Treatment of *Nitella* for a short time with .01 M NaCl[21] reduced the rate of penetration, when the cells were subsequently exposed to cresyl blue, by about 50 per cent. This effect of NaCl could be removed by rinsing the cells in a solution of $MgCl_2$ or $CaCl_2$ before exposing to the dye. In models, the addition of salicylic acid greatly reduced the penetration of cresyl blue but not of phenol red (66).

ACCUMULATION

Nature of accumulation. It is customary to speak of accumulation when, for example, the concentration of K^+ becomes higher inside than outside and it is often implied that energy is required to bring this about. But we know that if a system is moving toward a Donnan equilibrium (*i.e.*, with a rundown of energy) potassium may be entering and reaching a much higher concentration inside than outside.

It would seem logical to reserve the term "accumulation" for cases where an expenditure of energy is required, *i.e.*, where the chemical potential of a compound rises to a higher level inside than outside. In that case, we might speak of the accumulation of KCl but refrain from speaking of the accumulation of K^+ as being ambiguous from the standpoint of thermodynamics.

The fact that K^+ can reach a much higher concentration inside than outside is evident from Tables I and II. At one time it seemed natural to try to account for such cases by means of the Donnan equilibrium but this is impossible in the case of the cells just referred to.

In the first place, the chemical potential of KCl, which is proportional to the ionic activity product [K] [Cl], is, in most cases, much higher inside the cell than outside (Table II) (in the Donnan equilibrium it is equal on both sides).

In the second place, the ratios of the various ions inside to those

[21] This effect is apparently not produced by treatment with distilled water.

TABLE I

Chemical Analyses of Sap (The numbers in parentheses denote percentage when Cl is taken as 100)

	Sea water[1]	Sap of *Valonia macrophysa*[1] (Bermuda)	Sap of *Valonia ventricosa*[2] (Florida)	Sap of *Halicystis Osterhoutii*[3] (Bermuda)	Sap of *Nitella clavata*[4] (California)	Pond water bathing *Nitella clavata*[4]	Sap of *Chara ceratophylla*[5] (Finland)	Brackish water bathing *Chara ceratophylla*[5]
	M	M	M	M	M × 10³	M × 10³	M × 10³	M × 10⁵
Cl*	0.580	0.597	0.608	0.603	90.8	0.903	225.0	73.0
	(100.00)	(100.00)	(100.00)	(100.00)	(100.00)	(100.00)	(100.00)	(100.00)
Na	0.498	0.09	0.0348	0.557	10.0	0.217	142.0	60.0
	(85.87)	(15.08)	(5.73)	(92.4)	(11.0)	(24.0)	(63.1)	(82.2)
K	0.012	0.5	0.576	0.0064	54.3	0.051	88.0	1.4
	(2.15)	(86.24)	(94.74)	(1.01)	(59.8)	(5.6)	(39.1)	(1.9)
Ca	0.012	0.0017	Trace	0.008	10.2	0.775	5.3	1.8
	(2.05)	(0.285)		(1.33)	(11.2)	(85.8)	(2.4)	(2.5)
Mg	0.057	Trace?	Trace	0.0167	17.7	1.69	15.5	6.5
	(9.74)			(2.77)	(19.5)	(187.0)	(6.9)	(8.9)
SO_4	0.036	Trace?	Trace	Trace	8.33	0.323	3.9	2.8
	(6.26)				(9.2)	(35.8)	(1.8)	(3.9)
H_2PO_4	—	—	—	—	3.61	0.0002	4.1	Trace
					(4.0)	(0.02)	(1.8)	
NO_3	—	—	—	—	0	0.55	0.4	0.005
					(0)	(60.8)	(0.18)	(0.007)
				pH**				
	8.	5.8	5.6–6.0†	5	5.2	7.2	5.9††	7.9

For analyses of *Valonia* at Naples (which are not in complete agreement) see 89, 39, 112, 113, 20. S. C. Brooks (16) finds in *Halicystis ovalis* (Lyng.) Areschoug of the Pacific coast the ratio K ÷ Na = 1.5; see also 52. S. C. Brooks (20) calls attention to differences in the K ÷ Na ratio of different species in different localities. It should be remembered that there may be considerable difference in the same species in the same locality. Thus, in Bermuda the K ÷ Na ratio in *Valonia macrophysa* Kütz varies from 2.55 to 5.72 (77; 97, 370). The sap of *Valonia macrophysa* contains 1.4 parts per thousand of organic matter and that of *Chara ceratophylla* less than 30 parts per thousand (28).

[1]*Cf.* 92, where "parts per thousand" of Ca in the sap, as given, is 10 times too large. See also 15. [2]Cooper and Blinks (29). The figures are here corrected for slight evaporation. This species is presumably *V. ventricosa* J. G. Ag. [3]Blinks and Jacques (7) (the figure for total gram equivalents of cations should be given as 0.613). Blinks (2). [4]Zscheile (128), figures revised from Hoagland and Davis (45) which see for other analyses; also (47). [5]Collander (28).

*Including Br calculated as Cl. **Except in the case of sea water the values given represent a rough general average; the determinations are doubtful because the sap is but slightly buffered. †M. M. Brooks (12); M. Irwin (unpublished results). ††No allowance for salt error.

TABLE II

Ratio of Internal to External Concentration

(Conc. in sap ÷ conc. in surrounding medium)

	Valonia macrophysa (Bermuda)	*Valonia ventricosa* (Florida)	*Halicystis Osterhoutii* (Bermuda)	*Nitella clavata* (California)	*Chara ceratophylla* (Finland)
Cl	1.03	1.05	1.04	100.50	3.1
Na ...	0.18	0.07	1.12	46.10	2.4
K	41.6	48.0	0.53	1065.00	63.0
Ca ...	Very small	Very small	0.67	13.17	2.9
Mg ..	Very small	Very small	0.29	10.47	2.4
SO_4 ..	0	0	0	25.80	1.4
H_2PO_4	——	——	——	18050.00	> 400.0
NO_3 ..	——	——	——	0	80.0
H* ...	158	158	1000	100	100

* The values increase when photosynthesis raises the pH just outside the protoplasmic surface.

outside do not in the least correspond to the Donnan equilibrium (Table II).

In the third place, we find in the case of *Valonia macrophysa,* for example, no indiffusible ions in sufficient concentration to bring about such great differences. It is true that Donnan ratios might occur in the absence of indiffusible ions, as pointed out by Teorell (122, 123, 125), given a sufficient potential due to outward diffusion of an electrolyte. Evidently this does not happen in the cells here considered for they show no Donnan ratios, as is evident from Table II (99, *981*).

Moreover, equilibrium is impossible as long as metabolism and growth continue. In place of an equilibrium we have in these cells a steady state. So long as they are growing, water and electrolytes enter in a fixed ratio so that the composition of the sap remains much the same while its volume increases. Hence the substance which penetrates most rapidly is the one which predominates in the sap (99, *980*).

Internal concentration probably depends, in many cases, on the fact that the cellulose wall restricts the entrance of water, thus favoring increase in concentration of electrolytes inside. As the cell matures, growth and the production of carbon dioxide fall off and changes in permeability may tend to prevent the egress of sub-

stances already accumulated; perhaps this happens in the case of human erythrocytes which appear to allow no potassium to pass out. Regarding models of mature cells see 81.

Accumulation in models. Accumulation occurs in models (modified from those described by Irwin) consisting of an aqueous solution *C* representing the sap, a non-aqueous layer *B* representing the protoplasm, and an external aqueous solution *A* (Fig. 3). *A*

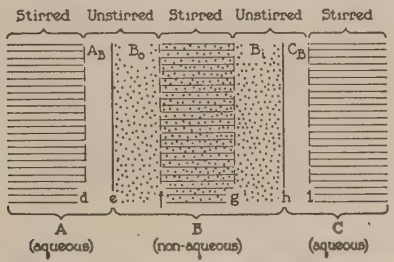

FIG. 3. Diagram of layers in the model. The aqueous phase *A* has an unstirred layer which is represented between *d* and *e*; from *e* to *f* is the corresponding unstirred layer in the non-aqueous phase *B*. Similar layers are present at the boundary between the non-aqueous phase *B* and the aqueous phase *C*.

contains KCl and is more alkaline than the sap (as is the case in *Valonia* and many other living cells); *B* consists of guaiacol + *p*-cresol (called G. C. mixture); for convenience we call them, collectively, H*G*. *C* contains, at the start, distilled water through which bubbles CO_2 to imitate its production by the living cell.

Potassium passes through the non-aqueous layer and enters the artificial sap where its concentration becomes much higher than in the external solution. It first combines at the outer surface according to the equation $KOH + HG \rightleftarrows KG + H_2O$. On reaching the artificial sap we have the reaction $KG + H_2CO_3 \rightleftarrows KHCO_3 + HG$. In time, a steady state is set up in which water and electrolyte enter in a fixed ratio so that the volume of the "artificial sap" increases while its composition stays nearly constant (this seems to be similar to what happens in living cells).

Evidently H*G* remains constant in amount and merely acts as a carrier. From a thermodynamic standpoint we have an exchange of K^+ in the external solution for H^+ in the artificial sap. But from a kinetic standpoint such a picture is entirely misleading, for

when KG moves through the non-aqueous layer it does so chiefly in molecular form, as the dielectric constant is too low to permit much dissociation. According to Shedlovsky and Uhlig (116), the dissociation constant of KG in guaiacol is about 5×10^{-5}.

This seems to resemble what happens in *Valonia*. The models furnish other important analogies (81, 98, 107, 108, 109, 110, 111). In the model, as in *Valonia*, the order of penetration* is $K > Na > Mg > Ca$; similar differences between the external and internal solutions are found in both in respect to pH, free energy, etc. (*cf.* 111). Similarities exist in respect to ionic mobilities (116) and potential (102*a*). Furthermore, bases enter by combining but this does not apply to acids.

We may say that when the internal activity product [K] [Cl] becomes greater inside a living cell than outside, KCl accumulates and energy is required for this process. This must be furnished by chemical activity.[22] Actually, the internal product [K] [Cl] is greater than the external in *Valonia* (Table I), in *Nitella* (45, 47), in *Chara* (28) and in many other living cells (72, 73).

The models likewise have chemical activity which can make the product [K] [Cl] greater inside than outside. To show that KCl can penetrate we place distilled water in C (with no CO_2 bubbling) and .1 M LiCl and .05 M KOH or .05 M KG in A. We find that these substances pass into C so that in the course of time A and C would become identical in composition. To hasten this we empty C and fill it with solution taken from A. We then start CO_2 bubbling in C and presently the concentration of potassium begins to increase; this continues until it becomes about four times as great as in A. The ionic concentration product (K) (Cl) is then about four times as great in C as in A. The ionic activity product [K] [Cl] is a little less because the activity coefficients are somewhat smaller in C than in A owing to the difference in ionic strength (108).

The energy necessary for this marked rise in the chemical potential of KCl (which is proportional to the product [K] [Cl]) in C

* This is the order of penetration through the body wall of a holothurian (81*a*, 81*b*, 81*c*, 81*d*).

[22] In the model, K^+ can reach a much higher concentration inside than outside without raising the chemical potential of KCl above that outside. This is because K^+ enters much more rapidly than Cl^- so that, for example, when K^+ is four times as concentrated inside Cl^- is less than one-fourth as concentrated in which case the product [K] [Cl] is less than outside.

is derived from the reactions occurring in the system and from the continual renewal of the solution in *A* and from the supply of CO_2.

Evidently the system has sufficient energy to raise the chemical potential of KCl to a higher level inside than outside just as the living cell does. In the cell both K^+ and Cl^- can reach a higher concentration inside.[23] But in the model Cl^- does not behave thus. In this respect it differs from K^+, for K^+ enters as *KG* which moves rapidly through *B* but passes out as $KHCO_3$ which moves slowly. The cell apparently has a device for making Cl^- move out more slowly than it moves in. Our imitation would be more complete if we could introduce this into the model, *e.g.*, by finding a substance which takes up more KCl as the pH rises (for the rôle of partition coefficients see p. 301). If, for example, a substance were employed that takes up more water at higher pH, it might take up more KCl, since it often happens that the more water a non-aqueous substance contains the more inorganic electrolyte it will take up from an aqueous solution.

The fact that accumulation depends on a relatively rapid inward movement has been emphasized by Irwin who shows that it is aided by the difference in partition coefficients at the inner and outer protoplasmic surfaces (61, 62, 64, 65, 67).

It is easy to make a model in which K^+ goes in against a concentration gradient or a different model in which Cl^- goes in against a concentration gradient but whether both these things can happen in the same model remains to be seen.

Since the cell has energy at its disposal, it is not surprising that KCl can accumulate even when no potassium compound seems to have an excess of chemical potential outside. This appears to happen in *Nitella* under certain conditions (80, 100). In this respect *Nitella* differs from *Valonia* where KOH has an excess of chemical potential outside. But *Nitella* requires no more energy to produce a given excess of chemical potential of KCl inside than does *Valonia*. The chief difference is that in the case of *Valonia* the first step in the process may be the entrance of KOH, but there is no evidence of this in *Nitella*. The energy necessary for accumulation is derived from metabolism and it has been suggested by Steward and Berry (121) and by Hoagland and Broyer (44) that

[23] In *Valonia* the concentration of Cl^- is only slightly higher inside, *i.e.*, .6 M inside and .58 M outside. See Table I, p. 295. In *Nitella* the activity of Cl^- is much higher inside than outside.

certain kinds of metabolism are of especial importance in this connection.

Although the cell has a supply of energy by which it can raise chemical potentials (*e.g.,* that of KCl) to a higher level inside than outside and can cause both K^+ and Cl^- to reach a higher concentration inside (Table I), the steps by which this is brought about need investigation. The cell does not use its energy to bring about such a result with all substances. In fact, with *Valonia* only potassium and ammonium compounds are normally so treated. This is because of their rapid penetration. It would seem that the sodium, magnesium and calcium compounds penetrate slowly as compared with water so that the internal chemical potential does not rise as high as it otherwise would. Cells which have ceased to grow might show a different situation but such cells may have a different metabolism and may possibly become less permeable to certain substances.

The ammonium compounds are of especial interest. Although the chemical potential of NH_4OH (or the internal product $[NH_4]$ $[OH]$) did not in any case become greater in *Valonia* sap[24] than outside[25], the product $[NH_4]$ $[Cl]$ in some cases became more than 100 times as great inside (31, 77). To judge from the data of Irwin (55) a similar situation obtains in *Nitella* with brilliant cresyl blue for, although the chemical potential of the undissociated dye base does not become greater inside than outside, that of the dye chloride does, since both the dye cation and Cl^- reach much higher concentrations inside than outside.

It is significant that the energy of the cell is not applied to bring about an accumulation of non-electrolytes. This would seem to indicate that it acts electrically. This is also the case with the models hitherto studied.

It may be added that those who regard the entrance of electrolytes as due chiefly to ionic exchange may not subscribe to the views here set forth. Regarding this see 8, 8*a,* 8*b,* 15, 17, 21, 38*a,* 50, 51, 68, 90, 91.

Let us now turn to the important problem of selective permeability.

[24] Osterhout, W. J. V. and Cooper, W. C., Jr., unpublished results.
[25] This is true also of *Halicystis* (*cf.* 2). For *Nitella* see 54.

SELECTIVE PERMEABILITY

Selective permeability is well illustrated by the large cells employed in these studies. For example, *Valonia macrophysa* takes up about forty times as much potassium as sodium from the sea water although sea water contains relatively little potassium. In order to clarify the problem let us consider the chief factors involved.

When the sap of a growing cell remains approximately constant in composition it is evident that water and electrolyte must enter in a fixed ratio so that a steady state obtains in which the composition of the sap depends on the rate of absorption of its various components.

As an example we may take the penetration of sodium and potassium on the assumption that they enter as hydroxides, as stated above (p. 288). As a first approximation we may neglect the effect of their chemical reactions with the protoplasm since if their behavior in this respect is similar it will not greatly affect the comparison, especially at lower concentrations (100). Hence we may write as an approximation

$$\frac{R_{KOH}}{R_{NaOH}} = \frac{P_{KOH}\,([K_o]\,[OH_o] - [K_i]\,[OH_i])}{P_{NaOH}\,([Na_o]\,[OH_o] - [Na_i]\,[OH_i])}$$

where R_{KOH} is the rate of entrance of KOH, P_{KOH} is the permeability of the protoplasm to KOH, and the subscripts o and i refer to activities outside and inside, respectively. Substituting numerical values, we obtain $P_{KOH} \div P_{NaOH} = 331$ (*99*, *991*); that is to say, the protoplasm is 331 times as permeable to KOH as to NaOH.

What causes this great difference? Presumably the rate of entrance depends chiefly on two factors, namely:

(1) The diffusion constants in the protoplasm. If the entering electrolyte forms a compound with a constituent of the protoplasm the diffusion constant of this compound must be taken into consideration.[26] It is evident that we can not expect sufficient difference in the diffusion constants to account for this result.

(2) The concentration gradients in the protoplasm. These depend on the partition coefficients.[3] This may be illustrated by the use of models (Fig. 4).

[26] The entering electrolyte may form one compound in the non-aqueous and another in the aqueous layer of the protoplasm.[9]

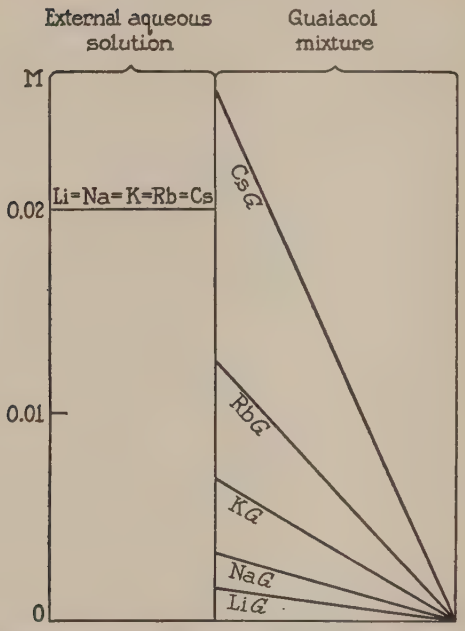

FIG. 4. Shows concentration gradients in the outer unstirred layer of the non-aqueous layer (B_o in Fig. 3) of the model (this is assumed to be thin enough to make the concentration gradients approximately linear).

In the external solution the concentrations are all .02 M and it is assumed that two salts are present in every case (*e.g.*, Na + K or Na + Cs, *cf.* 110). Evidently, when Na*G* and K*G* are present, the concentration gradient is about twice as great for K*G* as for Na*G* and it is found that the rate of entrance is correspondingly greater (110).

The order of penetration is that of ionic mobilities in water but as all these salts are very weak electrolytes in the guaiacol mixture they move through it chiefly in undissociated form. This is doubtless true of living cells.

When we place in the outside solution (*i.e.*, in *A*, Fig. 3) .02 M KOH + .02 M NaOH (or the corresponding concentration of K*G* and Na*G*) the concentrations are equal. But they are not equal in the non-aqueous layer *B* (in which diffusion is so slow that it controls the rate of entrance). Here the partition coefficient for K*G* is .35 and for Na*G* is .165 (110). Hence the concentration gradient across *B* at the start is .35(.02) = .007 for potassium and .165(.02) = .0033 for sodium. As the concentration gradient is

much greater for potassium than for sodium we might expect a much higher rate of entrance for potassium (84). This is actually the case (109, 110).

According to Shedlovsky and Uhlig (117), the partition coefficient is a function of the ionic radius and we should, therefore, expect the rate of entrance to increase in the order Li > Na > K > Rb > Cs, as is actually the case (110). Since it happens that this is also the order of ionic mobilities in water it might be considered a proof of entrance by ionic exchange did we not know that these are weak electrolytes in *B* (116) so that they move through it mostly in undissociated form (Fig. 4).

The partition coefficient of a compound will increase as the partition coefficients of the ions increase. Moreover, as the dielectric constant of the non-aqueous layer decreases the dissociation constant will follow suit and the proportion of undissociated molecules will become greater. With ammonia (p. 286) we have to do with NH_4X. If X^- is indiffusible we have the situation discussed by Teorell (125); this may not have much effect on the rate of entrance which depends chiefly on undissociated molecules.

Similar considerations doubtless apply to living cells for potassium usually enters more rapidly than sodium (but the difference may be much greater than is indicated by the equation of Shedlovsky and Uhlig and here chemical combination may play a rôle as already suggested on p. 286).

A similar dependence on partition coefficients appears when we compare the entrance of a weak acid and its salts. Thus the partition coefficient of molar acetic acid between water and guaiacol is about 30 times as large as that of molar potassium acetate.[27] Hence

[27] Unpublished results. This is to be expected on theoretical grounds. This may be illustrated by the following hypothetical case (the figures denote activities).

Aqueous	Non-aqueous
$(H)\ (A) = k\ (HA)$	$(H)\ (A) = k'\ (HA)$
$10^{-5}\ 10^{-4} = 10^{-6}\ 10^{-3}$	$10^{-6}\ 10^{-5} = 10^{-10}\ 10^{-1}$
$(Na)\ (A) = k_s\ (NaA)$	$(Na)\ (A) = k'_s\ (NaA)$
$9(10^{-5})\ 10^{-4} = 1\ \ 9(10^{-9})$	$9(10^{-6})\ 10^{-5} = 10^{-4}\ 9(10^{-7})$

Here we put the activity partition coefficient of the undissociated acid (HA) and that of the undissociated salt (NaA) the same, following the scheme of Lewis and Randall (Lewis, G. N., and Randall, M., Thermodynamics, New York, 1923, p. 262; see also Shedlovsky and Uhlig (116)). NaA is, of course, the stronger electrolyte in the non-aqueous.

If we now make the very reasonable assumption that the activity coefficients of undissociated NaA and of undissociated HA in the non-aqueous do not differ greatly the concentration of NaA in the non-aqueous will

the rate of penetration of the free acid is greater just as with living cells.

Regarding bases it may be said that in the models KOH penetrates more rapidly than KCl because KOH forms *KG* which has a higher partition coefficient than KCl and this appears to apply to *Valonia* (p. 290).

Regarding the penetration of dyes, see the papers of Irwin and of M. M. Brooks (bibliography in 99). Nirenstein (91*a*) and Irwin (66) found that both acid and basic dyes could enter simultaneously in certain models. It seems probable that the peculiarities of various cells may be imitated by means of models.

When substances are compared whose partition coefficients are similar, the order of penetration will be determined by their molecular weights. This seems to be the case with certain non-electrolytes (115, 118).

To recapitulate, we may say that selective permeability is determined chiefly by diffusion constants and concentration gradients and that the latter depend upon partition coefficients. The importance of partition coefficients was recognized by Overton but he considered only those at the outer phase boundary of the protoplasmic surface layer. Irwin has shown that the partition coefficients at the inner phase boundary of this layer must also be considered (64) (also papers cited in 99), and where a vacuole exists its surface layer must be taken into account (67).

The diffusion constants and partition coefficients are not constant but depend on temperature, concentration (110, 117), the presence of other substances, electrolytes and additional factors. Hence the permeability of the protoplasm varies.

With the aid of the facts set forth in the preceding sections we may now try to form a picture of the protoplasmic surface in these cells.

NATURE OF THE PROTOPLASMIC SURFACE

It behaves as a liquid. This is evident when protoplasm is squeezed out of these cells and comes into contact with water; it then rounds up like an oily liquid. According to Chambers (23), the surface of certain marine eggs also behaves as an oily liquid.

be very small and the concentration in the non-aqueous divided by that in the aqueous phase will be much greater in the case of HA than in that of NaA (and still more so if HA associates in the non-aqueous to form double molecules).

If the surface is liquid it cannot be a mosaic in the sense of Höber.[28]

In many cases, the protoplasm is normally covered with a layer of something analogous to the cellulose wall of plants (*e.g.*, chitin or cellulose) so that in experiments on cataphoresis or on wetting, we may be dealing with such materials rather than with the true protoplasmic surface. The protoplasm in contact with the vacuole of the cell is probably not covered in this way; its surface acts like an oily liquid at all times.

It is non-aqueous. As already stated, this is shown by a variety of evidence (99) and especially by electrical measurements. An example of the latter is seen in *Nitella*. A spot in contact with .01 M NaCl is 85 mv. positive to one in contact with .01 M KCl. This would not be possible with an aqueous gel, *e.g.*, protein imbibed with water (94).

The behavior of many substances, including weak acids and their salts,[27] would not be explainable on the basis of an aqueous surface (see p. 303).

Its thickness. It must be thick enough to account for the very slow inward diffusion of many substances which would enter rapidly in the absence of a non-aqueous layer. For example, salts enter very much more slowly than alcohol (*cf.* 99, *1010*).

It seems doubtful whether a layer only one or two molecules thick could account for this situation. A layer of this thickness could not suffice in the case of rapid increase in cell surface nor of chemical combination between the surface and the entering electrolyte, which appears to happen in the case of penetrating bases (p. 286). Nor would it account for the great changes which can be experimentally produced in the surface layer. These will be considered more in detail in the next section.

It is not homogeneous. It cannot be homogeneous when profound modifications of the surface are possible which are reversible in character. For example, in *Nitella* one of the most striking properties of the protoplasmic surface is the ability to distinguish between sodium and potassium; in this respect it acts almost like a potassium electrode (this is known as the potassium effect).

[28] Briggs (8) and Söllner (118) state that such a mosaic would not admit both anions and cations as supposed by Höber. See also S. C. Brooks (21).

When we lead off from .01 M KCl to .01 M NaCl on the surface of the cell we obtain 85 mv. from which we calculate that the apparent mobility of K^+ is about 40 times that of Na^+ (94). All this disappears when the cells are washed for several days in distilled water (105). We then find that the water contains substances which can be extracted from it with petroleum-ether and which restore the potassium effect when redissolved in water and applied to the surface (40, 106). Hence it is evident that an organic substance is dissolved out by the distilled water which is responsible for the potassium effect. For convenience, this substance or group of substances may be called *R*. The nature of *R* is unknown but it has been found that the potassium effect can be restored by such substances as NH_3 (101) and adrenalin (106); this does not seem to be primarily a question of alkalinity for such bases as aniline, toluidine, and alkaloids do not restore the potassium effect.

It would seem that we might imitate the non-aqueous protoplasmic surface by taking an indifferent substance and dissolving guaiacol in it. The guaiacol would enable it to distinguish electrically between potassium and sodium (though not to such an extent as the *Nitella* cell). In contact with distilled water the guaiacol would come out and leave the indifferent substance which would be unable to distinguish between them.

Cells of *Nitella* behave very much like nerve fibers in that they can be stimulated electrically to give action currents. The irritability disappears after exposure to distilled water (103); it thus acts like the potassium effect and can be restored in somewhat the same way so that it probably depends on the presence of a substance or a group of substances.

Another illustration is seen in the effect of guaiacol on *Valonia* which changes the order of apparent ionic mobilities in the surface from $K > Cl > Na$ to $Na > Cl > K$ (102).

CONCLUSION

The facts and principles here set forth are the results of investigations of large cells which offer special advantages for such studies. How far they are applicable to other cases remains to be seen.

This brief outline indicates progress in dealing with some very

interesting variables. It also gives a hint of the host of problems awaiting solution.

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EXPLANATORY NOTES

BY THE EDITORS

Adapted, in part, from Hackh's "Chemical Dictionary"

acid: a chemical compound which yields hydrogen ions, H^+, when dissolved in water or a compound whose hydrogen can be replaced by metals or basic radicals or which reacts with bases to form salts and water.

acid, strong: an acid that ionizes greatly.

acid, weak: an acid that does not ionize greatly.

activity coefficient: a factor by which a concentration is multiplied to obtain a value called the activity which can be used in place of the actual concentration in equations set up for ideal solutions, *i.e.*, solutions in which the ions or molecules of the solute have no more influence upon each other than they would have at infinite dilution (where the activity coefficient is unity). For very dilute solutions of univalent ions at 25° we have $-\log \gamma = 0.506 \sqrt{C}$, where γ is the activity coefficient and C is the molar concentration.

anion: see ion.

base: a compound which yields hydroxyl ions, OH^-, in aqueous solution or a compound which reacts with an acid to form water and a salt.

cataphoresis: the motion of electrically charged particles suspended in a medium under the influence of an electric field.

chemical potential: the free energy per mole of a compound under constant conditions. The chemical potential of KOH in an aqueous solution is proportional to the [K][OH], where the bracket denotes activity (*i.e.*, the concentration multiplied by the activity coefficient).

concentration: the strength of a solution. Molar concentration is the number of moles of a substance dissolved in one liter of solution and a mole is a gram-molecule, *i.e.*, a quantity of matter weighing M where M is the molecular weight.

concentration gradient: in a layer of solution with a depth x and a concentration C_1 at the top and C_2 at the bottom, the concentration gradient is $\frac{C_1 - C_2}{x}$. Strictly speaking, this applies only when the gradient is uniform.

dielectric constant: a dielectric is an insulator or non-conductor of electricity. The force exerted between any two charged point sources q_1 and q_2 is $F = \frac{q_1 q_2}{Dd^2}$, where d is the distance separating them and D is the dielectric constant (constant for any particular medium at constant temperature). Hence the attraction between Na^+ and Cl^- is less in water ($D = 81$) than in guaiacol ($D = 14$) so that NaCl is a strong electrolyte in water and a weak electrolyte in guaiacol.

diffusion constant: the constant D in $S = -Dqt\ \frac{C_1 - C_2}{x}$, where S is the weight of substance diffusing in t seconds through a cylinder of length x and cross-section q, and $\frac{C_1 - C_2}{x}$ is the concentration gradient, *i.e.*, the difference between the concentrations at opposite ends of the cylinder divided by its length. Strictly speaking, this applies only when x is infinitely small: otherwise, it is only an approximation which becomes more exact as x becomes smaller.

Donnan equilibrium: a membrane equilibrium involving an unequal distribution of ions which are freely diffusible through a membrane when it has on one side ions which do not diffuse.

dissociation: the breaking apart of a molecule into ions by physical means.

dissociation constant: the product of the molar concentrations of the different participating ions divided by the molar concentration of undissociated molecules. For acids it is usually stated thus $K = \frac{(H^+)(X^-)}{(HX)}$ where (H^+) and (X^-) represent the molar concentrations of the ions and HX represents the molar concentration of the undissociated portion (X represents any anion that may accompany the hydrogen ion).

electrolyte: any substance which dissociates into two or more ions, to a great or small extent, when dissolved in water or other solvent. Solutions of electrolytes thus conduct the electric current.

hydroxide: as here used signifies a compound yielding OH^- on dissociation: example, KOH.

ion: an electrically charged atom or more complex structure; cation if positively charged, as H^+; anion if negatively charged, as Cl^-, since they travel in solution to the cathode or anode, respectively.

ionization: the breaking up of a molecule into two or more negatively and positively charged components or ions.

micron: one millionth of a meter.

mole: see concentration.

pH = the symbol for the logarithm of the reciprocal of the hydrogen ion activity (which becomes almost equal to the concentration in very

dilute solutions). Values indicate degrees of acidity or alkalinity: pH 7 is neutral, higher values more alkaline, lower values more acid.

partition (distribution) coefficient: the concentration of a substance in one phase divided by its concentration in another phase in equilibrium with it, *e.g.*, S_w/S_F may be the coefficient where S_w = the concentration of a substance in water and S_F = its concentration in chloroform when the three components are shaken together until equilibrium is reached.

Q_{10}: a symbol whose value indicates how much the speed of a chemical reaction is multiplied for each 10° C. rise in temperature.

salts: substances resulting from reactions between acids and bases: example, sodium chloride.

temperature coefficient: any factor that indicates quantitatively the effect of temperature upon a property of matter or upon a process.

time curve of the first order: a curve expressing a reaction of the first order, *e.g.*, when a substance A decomposes to form B. If the initial amount of A be called a and the amount of B be called x, we have $kt = 1n \frac{a}{a-x}$, where t is time and k is the velocity constant of the reaction. $a-x$ or x is plotted as ordinates and time as abscissae.

time curve of the second order: a curve expressing a reaction of the second order, *e.g.*, one in which two substances A and B unite to form C. If the amounts of A and B at the start are equal we may call this value a and the amount of C at any given time, t, may be called x. We then have $kt = \frac{x}{a(a-x)}$, where k is the velocity constant of the reaction. $a-x$ or x is plotted as ordinates and time as abscissae.

SOME ASPECTS OF THE CYTO-GENETICS OF OENOTHERA

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Oenothera has long been known as one of Nature's most consistent non-conformists. In many features of its genetical and cytological behavior it has shown a wide departure from the rules which prevail in other organisms. It occupies, therefore, a position of major interest in the cyto-genetic field.

It was de Vries who first discovered that *Oenothera* is peculiar. His attention was called to the genus in 1886 when he found *Oe. lamarckiana* growing in great numbers in an abandoned field near Hilversum, in Holland. He found, among thousands of typical plants, occasional aberrant or exceptional individuals which seemed to have arisen as sports from the prevailing type. Collecting seed from typical plants, he grew many thousands of individuals in his experimental garden over a period of years and found the same aberrant types appearing from year to year in small percentages that had been present in the field, some of which bred true to their new characters. All of this seemed to de Vries to furnish a clue to the method by which new species are formed; and it was principally on the basis of his extensive study of *Oenothera* that he was led to formulate the celebrated Mutation Theory of Evolution (101) which assumed that species spring into existence suddenly, as a result of genetical modifications of major importance, rather than through gradual accumulation of many small variations.

But de Vries soon found that *Oenothera* is peculiar, not only because it produces sports, but also because it shows in certain aspects of its ordinary breeding behavior an anomalous condition, setting it apart from other organisms. de Vries was already becoming familiar, through his own experiments on a wide variety of material (98, 99, 100, 101), with the basic principles of heredity which he was later to find had already been set forth in Mendel's work. Thus, he was able to appreciate the uniqueness of *Oenothera* in certain aspects of its genetical behavior. Briefly, the anomaly which he found in *Oenothera* consists in the fact that a species which, when inbred, behaves like a pure species in that it breeds

perfectly true to type, may, nevertheless, behave like a hybrid when crossed with another species, for it may either produce progenies which include more than one kind of individual, as hybrids do, or it may produce different types of progeny when used as male and female parent (102, 103, 104, 105, 106). If such a plant is not hybrid, how can it give splitting progenies? On the other hand, if it is heterozygous or hybrid, how can it breed true? The anomaly seemed all the greater to de Vries when he learned that in many cases the individuals, resulting from such a cross between species, themselves breed true when selfed. According to Mendelian principles, as understood by de Vries, one would expect pure races, when crossed with each other, to produce uniform hybrid progenies, and one would expect the hybrids thus produced to give splitting progenies. But in the case of *Oenothera,* apparently pure species give splitting progenies and apparently hybrid individuals breed true. This can be made clear by a diagram contrasting the behavior of Mendel's peas with that in a selected case in *Oenothera.*

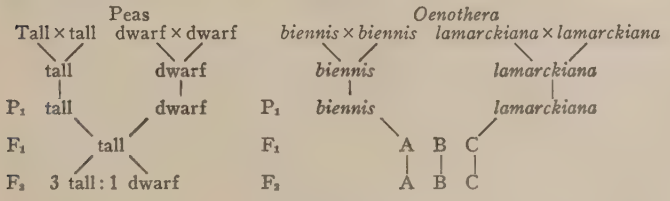

DIAGRAM 1

In peas, a pure tall strain, or a pure dwarf strain, remains true to type in an inbred line; and when these strains are crossed with each other, they produce a uniform F_1 because they themselves are homozygous. The hybrids thus produced, however, yield a splitting progeny in F_2, if inbred. In *Oenothera,* the species *biennis* and *lamarckiana,* for instance, breed true when inbred, just as do the tall and dwarf peas. When crossed as above, however, they produce, not a uniform F_1, but 3 distinct types of hybrid, and when these hybrids are selfed or inbred, each type, instead of yielding a splitting F_2, breeds essentially true, except in flower size (76).

This was a situation which de Vries was unable fully and correctly to explain, his failure being due, in part, to the fact that he

was long unwilling to consider *Oe. lamarckiana* in any other light than that of a pure species, realizing what effect admission of the heterozygosity of *lamarckiana* would probably have upon the interpretation of the nature of its mutants, and hence upon the whole mutation theory.

The complete analysis and explanation, from the genetical point of view, of this anomalous behavior, was the work of other investigators. Bartlett (1), Cobb and Cobb and Bartlett (5, 33, 34) in America, and Renner (59, 60, 74–82) in Germany, arrived independently at the concept of complex-heterozygosity, which was to form the starting point for our present adequate understanding of the peculiarities of *Oenothera* breeding behavior. Building upon this concept as a foundation, Renner and his followers, in an extensive and masterly series of studies, thereupon proceeded to establish the correctness of this concept and to elucidate further peculiarities as well. It is largely the work, therefore, of Renner and his followers (Oehlkers 69–73, Rudloff 84–86, Gerhard 54) which has given us the clear and complete picture which we now have of the genetical situation in *Oenothera.*

This situation will now be summarized, as follows:

(1) All oenotheras, so far as known, have 14 chromosomes. According to the ordinary rules of cytological and genetical behavior, we should expect to find them behaving as though they had 7 independent pairs of chromosomes in meiosis, and corresponding to these, 7 independent linkage groups. However, we find that a great majority of the spontaneous races of *Oenothera* which have been studied genetically behave as though they had but one pair of chromosomes and one linkage group.

A plant which had but one pair of chromosomes would receive one chromosome from each parent. The entire paternal set of genes would be in one chromosome and the entire maternal set in the other. In reduction division, therefore, the paternal genes would separate from the maternal, and one-half of the germ cells would be found to contain the entire paternal inheritance, the other half would contain the entire maternal inheritance (except for crossing-over). If the plant were highly heterozygous (and all species of *Oenothera* which behave in this fashion *are* highly heterzygous), the two sets of genes and the two types of germ cell

would differ materially. Furthermore, except for crossing-over, one would expect to find the genes in each chromosome sticking together generation after generation—there would be no more than 2 sets of genes in the germ cells of a given race, crossing-over excepted.

Now this is just the way in which most species of *Oenothera* behave. In these species, all (in some cases, not quite all) of the genes are united into a single linkage group—at least this is true of those genes which are not necessarily common to all species of *Oenothera.* All of the paternal genes of this group are separated in reduction division from all of the maternal genes, and there are produced but 2 kinds of germ cells, those containing the entire paternal set, and those containing the entire maternal set. These sets or "complexes," as Renner has called them, are passed on intact from generation to generation, and hence maintain their identity indefinitely. They are thus in a sense as much entities as are the species themselves; and hence Renner has given them names, such as *velans* and *gaudens,* making up *lamarckiana,* or *rigens* and *curvans,* making up *muricata.* Each species is a heterozygote, composed of 2 complexes; or in the terminology of Renner, it is a complex-heterozygote. But here is where the anomaly is found—*Oenothera,* while it acts in most cases as though it had but one pair of chromosomes (since it has but one linkage group), has in reality 14 chromosomes.

(2) All species in which the genes are mostly or entirely confined to a single linkage group, are highly heterozygous. In spite of this, however, they breed true, as de Vries originally observed. This is due to the presence of balanced lethals, which may be of 2 sorts: (a) the first sort prevent the development or functioning of those eggs or sperms, as the case may be, which carry a given set or complex of genes. These are sometimes called gamete lethals, although they probably function, not in the gametes proper, but in the gametophyte generation which precedes gamete formation or even in the spores which produce the gametophytes. They may do nothing more, in some cases, than inhibit pollen tube growth or prevent the microspores in which they are found from competing successfully in embryo sac development. (b) The second sort, the so-called zygote lethals, prevent the development of individuals which have received the same complex from both

parents. The result of either kind of lethal is to make only one combination of complexes possible in any given generation of an inbred line, namely, that which duplicates the previous generation; and hence the race, although heterozygous, is forced to breed true. (see diagram 2).

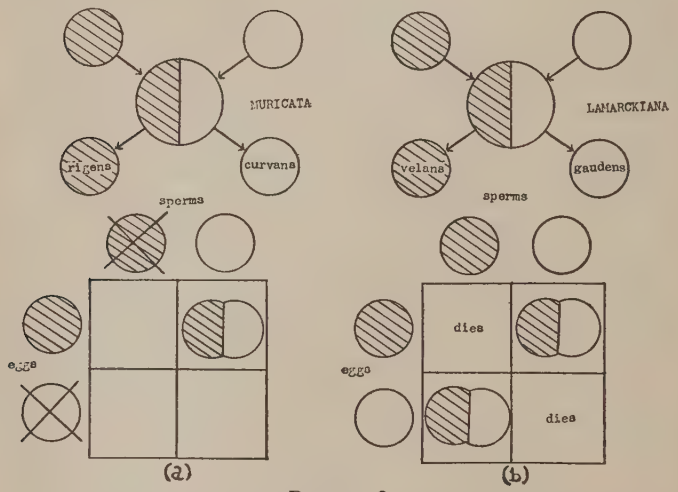

DIAGRAM 2

(a) *"Gamete lethals" in muricata. Rigens* is prevented by a lethal from functioning as sperm; *curvans* is prevented from functioning as egg. Hence only one combination is possible in each generation, *rigens · curvans.*

(b) *Zygote lethals in lamarckiana.* Both *velans* and *gaudens* function both as sperm and egg; but *velans · velans* and *gaudens · gaudens* die because these complexes possess each a zygote lethal. Hence only *velans · gaudens* survives.

(3) Although complex-heterozygotes breed true when selfed, and thus act as though they were pure, they show their heterozygosity when crossed with other species which have other lethals. Those species which, like *lamarckiana* or *grandiflora,* produce 2 kinds of functional sperms and 2 kinds of functional eggs, tend to produce twin or multiple types in their progenies; those which, like *muricata* or *chicaginensis,* produce but one kind of functional sperm and one kind of functional egg, produce unlike reciprocals. Some examples will make this clear (see diagram 3).

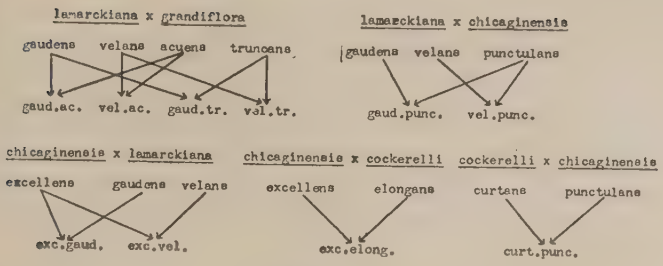

Diagram 3

(a) If one crosses *lamarckiana* with *grandiflora,* both parents having 2 types of sperms and 2 types of eggs, there will be 4 classes of progeny, thus showing the heterozygous character of both parents. (b) If *lamarckiana* is crossed with *chicaginensis,* only 2 classes of progeny will be formed, whichever way the cross is made, since *chicaginensis* has but one kind of functional sperm and egg. Since, however, its egg complex is very different genetically from its pollen complex, the 2 classes produced when *chicaginensis* is female parent will be very different from the 2 classes produced when it is used as male parent. Thus, the heterozygosity of *chicaginensis* is made clear by the production of unlike reciprocals, whereas the heterozygosity of *lamarckiana* is shown by the production of twins in both reciprocals.

(c) If *chicaginensis* is crossed with *cockerelli,* only one class of progeny will be found, whichever way the cross is made. The only clue to the heterozygosity of the parents is the fact that the reciprocal hybrids are unlike. These crosses alone, however, are not enough to tell whether both, or only one, of the parents is heterozygous.

In all these cases, one curious fact is to be noted, namely, that individuals receive inheritance from only 2 of their grandparents, and not from all 4, as is usual among other organisms. In the first example given, each individual has received, through each parent, either the entire inheritance which the latter has received from its mother, or the entire inheritance which it has received from its father; it has inherited from one paternal and one maternal grandparent only. In the other cases, individuals whose mother

is *chicaginensis* or *cockerelli,* for instance, can inherit, on the female side, only from the maternal grandmother. They cannot possibly receive inheritance from the maternal grandfather, except through crossing-over. On the other hand, if either *chicaginensis* or *cockerelli* is male parent, their progeny can inherit on the male side only from their paternal grandfather, and not from their paternal grandmother. This is a curious result of the linkage of all heterozygous genes into one group, coupled with the presence of balanced lethals.

Other examples might be cited to cover certain species which occupy an intermediate position between the *lamarckiana* type, with 2 kinds of functional sperm and egg, and the *chicaginensis* type, with but one kind of each. There are species, like *biennis* or *suaveolens,* in which both complexes can function as egg (one often but rarely), but in which only one complex is able to function as sperm. On the other hand, races are known in which the reverse is true; only one complex functions in the egg, but both may be transmitted through the pollen (the egg complex much less frequently than the other, however). Whatever the condition with respect to the appearance or non-appearance of one or both complexes in sperm and egg, however, the principle is illustrated by all that the various complex-heterozygotes, although they breed true when selfed, show their heterozygosity when outcrossed by the production of twin or multiple types, or of unlike reciprocals.

(4) The presence of lethals naturally leads to a considerable percentage of sterility. Forms with gamete lethals usually show considerable pollen sterility, inasmuch as one of the complexes is unable to function on the male side. Such forms, however, show little or no seed sterility when selfed, since all the functional eggs are capable of fertilization by any of the sperms, and consequently all zygotes are theoretically viable. On the other hand, complex-heterozygotes with zygote lethals show at least 50% seed sterility, since half the zygotes produced by inbreeding are homozygotes, and therefore inviable.

(5) We have seen so far that most species of *Oenothera* are highly heterozygous and are characterized by the union of all or nearly all of the genes, not common to all species, into a single linkage group, which group contains a balanced lethal system that prevents the production of homozygotes and thus enforces a condi-

tion of permanent heterozygosity upon these races. In view of this union of all or most of the genes in the various species, it is surprising, therefore, to discover that in the case of many *hybrids* between species, certain genes, which were linked together in the parents, are no longer linked in the hybrids—in other words, *the single linkage group in the parents has been broken up into two or more smaller ones in the hybrids.* In illustration, we may use certain data from the work of Renner (81) which are presented in diagram 4. At the top is listed a number of genes, and to the

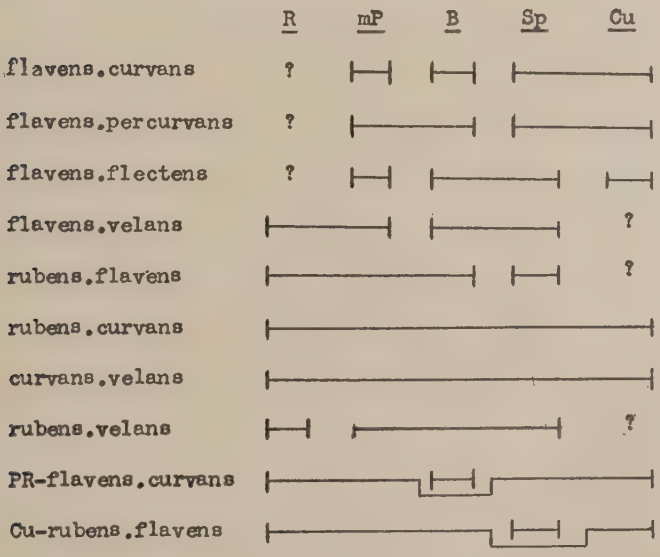

Diagram 4

left are various hybrid complex-combinations, obtained by crossing certain species. In *flavens · curvans* at least 3 linkage groups are present, with *mP* in one, *B* in another and *Sp* and *Cu* in the third. In *flavens · percurvans,* however, *mP* and *B* are in the same linkage group. In *flavens · flectens, Cu* is independent of *Sp,* the latter being linked with *B.* In *PR-flavens · curvans, B* is independent of the rest of the genes, which are all linked together. Similarly, as will be seen, the rest of the hybrids have each their own charac-

teristic number of linkage groups and distribution of these genes throughout the groups.

Now this is an utterly anomalous situation which cannot be understood on the basis of the conventional explanation of linkage, for in ordinary linkage, genes which are in the same linkage group at one time will always remain in this group, unless some accident, such as translocation or fragmentation, occurs, to alter the group. The alteration of linkage relations which occurs in *Oenothera,* however, is not in the nature of an accident, for it always occurs in a given way after a given cross, no matter how often the cross may be repeated, each hybrid having always its own characteristic number of linkage groups and distribution of genes throughout these groups.

The chief points have now been summarized at which *Oenothera* fails to abide by the ordinary rules of hereditary behavior. To recapitulate briefly: (1) although they have 14 chromosomes and should, therefore, have, theoretically, 7 independent linkage groups, most oenotheras behave as though they had but a single group. With respect to this one group, they are highly heterozygous and thus produce two and only two kinds of gamete from the standpoint of the gene complexes which are carried. (2) By means of balanced lethals, they manage to breed true when selfed; nevertheless, (3) the fact that they are highly heterozygous is shown in outcrosses. (4) The presence of balanced lethals results in a high degree of gametic or zygotic sterility. (5) When species are crossed, genes which were linked in the parent species often become independent in the hybrids between these species; there are in such hybrids two or more linkage groups in place of the original single one, and the number of linkage groups in a given hybrid is constant.

With this all-too-brief outline of the principal genetical peculiarities of *Oenothera,* we may next consider the question as to the physical basis of these phenomena. If the chromosome theory of inheritance is correct, it ought to be possible to find an adequate explanation of these peculiarities in terms of chromosome behavior.

Not all of the phenomena which have been mentioned above suggest unusual chromosome behavior. However, two of *Oenothera's* peculiarities are such as to raise doubts, to say the least, as to the normality of chromosome behavior in the genus. These are (1)

the linkage of all or most of the genes into a single linkage group, and (2) the alteration of linkage relations which takes place in species-hybrids. How are these to be explained?

The first of these phenomena may be explained in one of two ways. It is possible to assume, as one alternative, that all or most of the genes governing visible and contrasting characters in *Oenothera* reside in a single chromosome pair, that the other 6 pairs are almost or entirely empty of genes, or at the most carry only such genes as are common to all known gene complexes (91–95). According to this explanation, genetical linkage in *Oenothera* is no different from linkage in *Drosophila*—genes are linked because they reside in the same chromosome, and for no other reason.

This sounds like a plausible hypothesis but there are two objections to it. First, it is hard to understand how practically all heterozygous genes, and genes for visible differences between individuals and races, should come to lie within a single chromosome, and the same chromosome, in all these species. Secondly, this hypothesis is unable to account for the breaking up of the single linkage group which occurs in species-hybrids. It is obvious that genes which lie within a single chromosome must remain linked permanently, or until the chromosome becomes altered through fragmentation or translocation. They cannot reside in one chromosome in one generation and be scattered among a number of chromosomes in the next generation. Inclusion within a single chromosome cannot, therefore, be the basis for a type of linkage that varies from generation to generation.

The second alternative must, therefore, be considered, which is to the effect that the genes, apparently linked in *Oenothera* into a single large group, are actually scattered throughout the various chromosomes, but that there is some mechanism which ensures that all chromosomes of paternal origin will pass to one pole in the reduction division, and all those of maternal origin will pass to the other pole, thus resulting in the presence of the entire paternal gene complex in one-half the germ cells, and the entire maternal complex in the other half. This explanation seems at first sight even more bizarre than the other, but let us examine into chromosome behavior in the genus to see whether there is any ground for assuming that such a situation prevails.

It has been known since 1908 that the chromosomes of many oenotheras do not behave normally in meiosis. Gates (50–52) and Davis (39–41) showed that they have a tendency to remain in an unpaired condition, and to separate to the poles without having shown any marked association. It was not until later, however, (13–18, 42, 55) that it was realized that chromosome behavior in *Oenothera,* while often different from that found in other organisms, is, nevertheless, regular and consistent, following certain definite rules with great constancy. The situation, as it is now known to exist in those races which exhibit peculiarity, is quickly explained. In a large majority of the species which have been studied cytologically, most or all of the chromosomes, instead of pairing in late prophase of the first meiotic division, are found attached end to end to form closed circles, each circle having an even number of chromosomes (fig. 1). The number of chromosomes in a circle,

FIG. 1

FIG. 2

and the number of circles, is always constant in a given race. When metaphase sets in, the circles remain intact and are gathered as a unit into the center of the spindle. Adjacent chromosomes then begin to move in opposite directions—toward opposite poles (fig. 2). For a time after this movement begins, they hang suspended between the poles, the force toward the poles being apparently insufficient to cause the attached chromosomes to separate from one another. Then breakage occurs and the chromosomes are quickly separated. As a result, adjacent chromosomes pass, as a rule, to opposite poles. There are, of course, exceptions to this rule—accidents happen in a percentage of cases which alter the normal procedure. On the whole, however, adjacent chromosomes are separated to opposite poles in a very large majority of the cells.

This completes a brief outline of some of the genetical idiosyncracies of the oenotheras on the one hand and their cytological peculiarities on the other. The question now arises as to whether there is any causal relation between them. Can the linkage of all or most of the genes into a single linkage group be explained by the linkage of the chromosomes into chains? Can the alterations in linkage, which occur when crosses are made, be explained in terms of chromosome behavior?

It was early realized (14, 16, 18, 20, 21, 55, 72) that chromosome linkage could, indeed, explain the genetical peculiarities of the genus, provided one were to make a single assumption, namely, that chromosomes of paternal and maternal origin occupy alternate positions in the circle (diagram 5). It is easy to see that if they

DIAGRAM 5

Chromosomes of paternal origin (white) alternate with chromosomes of maternal origin (black) in the chain, and consequently are separated to opposite poles in reduction anaphase.

do occupy alternate positions, the separation of adjacent chromosomes to opposite poles, which normally occurs, would necessarily send all the paternal chromosomes to one pole, and all the maternal ones to the other; and thus one-half of the germ cells would have the complete paternal gene complex and one-half would receive the complete maternal set, which is what actually occurs in *Oenothera.*

This alternation of paternal and maternal chromosomes was, of course, a pure assumption at first; but fortunately, it was one that was capable of being tested. It was by this time becoming known that hybrids between species may have many sorts of arrangement of their chromosomes into circles and pairs (19, 23, 73). There are, as a matter of fact, 15 different arrangements possible in diploids with 14 chromosomes (see chart 1) all of which have now been found at least once among the many hybrids which have been studied. In some of these arrangements, the chromosomes are all or mostly in circles, in others they are mostly paired. Now if the extensive linkage of all or most of the genes in *Oenothera* is dependent upon the end-to-end union of the chromosomes in which the linked

genes reside, it is obvious that such linkage can last only so long as the chromosomes are thus united. Consequently, if we should cross two species in which the chromosomes, and hence the genes, were all united, and should find that the chromosomes were no longer united in the hybrid, but were mostly or entirely in a paired condition, then we should expect on the basis of this assumption to find the genes in the now separated chromosome pairs no longer linked —we should expect them to show independent assortment, and hence should look forward to a varied progeny if these hybrids were selfed. On the other hand, if the chromosomes in the hybrids were found to be united as they are in the parental races, we should expect to find the genes also still linked, which would be evidenced by the fact that such hybrids would breed true in following generations—would behave, in other words, just like the parental races. Obviously, then, the way to find out whether chromosome linkage results in extensive genetical linkage or not would be to cross species, determine the arrangement of chromosomes in the hybrids, and then grow the progenies of the hybrids to see whether a correlation exists between the degree to which the chromosomes are linked or free in the hybrids, and the degree to which the genes are linked or free.

The first study of this kind was made by Oehlkers (73) who showed that when *suaveolens* is crossed by *strigosa* (both species being true-breeding complex-heterozygotes) twin hybrids are produced, of which *albicans · stringens,* with ⊙[1] 12, breeds true, except for the independent splitting of one factor, whereas *flavens · stringens,* with ⊙ 4 and 5 pairs, shows independent segregation in respect to at least 3 factors. This study was followed later by an extensive series of tests, performed by Cleland and Oehlkers (31, 32) and Renner and Cleland (83). In these tests, some 50 different species-hybrids were grown, their chromosome configurations determined and their breeding behavior analyzed. This is not the place to review these studies in detail—it will suffice to emphasize the fact that the results were entirely confirmatory of the hypothesis that the linking together of the chromosomes, with paternal and maternal chromosomes alternating, and the separation of adjacent chromosomes to opposite poles, constitute the physical

[1] This symbol denotes a ring composed of the indicated number of chromosomes.

basis for the extensive genetical linkage found in *Oenothera*. Hybrids with a circle of 14 or a circle of 12 chromosomes were found to breed essentially or entirely true; at the other end of the scale, hybrids with 4 or 5 pairs gave progenies which showed much segregation, and those which occupied an intermediate position in the scale of chromosome configuration gave an intermediate amount of splitting in F_2. A close correlation was thus found between the number of chromosome groups present in a hybrid and the amount of splitting shown by the progeny of this hybrid. From these and subsequent studies (24, 30, 44, 47, 48, 49, 82, 86), it seems clear that the number of linkage groups in a plant depends, not upon the number of chromosomes or chromosome pairs, but upon the number of chromosome groups, whether these be circles or pairs. Thus, a plant with ⊙ 14 has but one linkage group, one with ⊙ 10 has one large and 2 small groups, etc. *Circles, then, form the basis of single linkage groups, just as pairs do, and* the *reason why a circle forms the basis of only one linkage group is that the paternal and maternal chromosomes in the circle alternate, and adjacent chromosomes go to opposite poles.*

In line with this evidence, is the evidence from the spontaneous races themselves. Some 54 distinct species or races have been studied cytologically up to the present. Of these, 22 have been proved to be complex-heterozygotes, whose genes are all, or nearly all, linked into a single group; and 19 are known to be races whose genoms are essentially similar, which carry no lethals, and which show independent segregation of their heterozygous genes. It is a striking fact that all 22 of the complex-heterozygotes have large circles, whereas all of those which are not complex-heterozygotes have mostly or entirely paired chromosomes. There is, therefore, in species as well as in hybrids, clear-cut evidence to show that extensive linkage is present only when the chromosomes are mostly or entirely linked, and absent when chromosome catenation is absent. The perfect correlation thus observed between genetical and cytological behavior cannot be purely a coincidence, for the number of forms which show it is much too great to allow of such an interpretation.

A further, and if anything, a more convincing proof of the causal connection between chromosome concatenation and extensive genetical linkage was furnished when it became known that the

union of chromosomes end-to-end in meiosis is a result of the phenomenon of segmental interchange. This realization, as we shall show, made it absolutely necessary to adopt the conclusion that paternal and maternal chromosomes alternate in the circle, and hence that the paternal and maternal gene complexes are segregated intact from each other in meiosis. It will be advisable at this point, therefore, to indicate briefly the nature of the proof that segmental interchange has been responsible for circle formation, and then to show that acceptance of segmental interchange as a fact makes it absolutely necessary to accept also the alternation of paternal and maternal chromosomes in the circle, and hence the causal relation between chromosome linkage and extensive genetical linkage.

The concept of segmental interchange (or reciprocal translocation) originated with Belling (2, 3) who adopted it to explain the presence of a circle of 4 chromosomes in a hybrid between two lines of *Datura stramonium.* The concept briefly is this: homologous chromosomes attract each other in synapsis, through some affinity which exists between homologous regions or, more specifically, between homologous genes. Now if a portion of one chromosome exchanges with a portion of another non-homologous chromosome, the pairing of homologous regions in synapsis will then result in the formation of a circle of 4. This will be made clear by a diagram in which homologous end-segments are indicated by corresponding numbers (diagram 6). Further interchanges between chromosomes in the circle and paired chromosomes will increase the size of the circle.

Belling (2) suggested that this process might explain circle formation in other genera, including *Oenothera;* and Håkansson (56, 57) and Darlington (35, 37, 38), taking up this suggestion, applied it more specifically to *Oenothera,* and showed that it was capable of explaining the situation in this genus. Meanwhile, Emerson and Sturtevant (46, 48, 97), as well as Blakeslee and the writer (4, 28, 29), were engaged independently in an effort to test the correctness of this hypothesis as applied to *Oenothera.* These authors realized that, if segmental interchange has occurred, each complex should have its own specific arrangement of end segments throughout the chromosomes, and thus, knowing the arrangements in certain complexes, it should be possible to predict the chromosome configura-

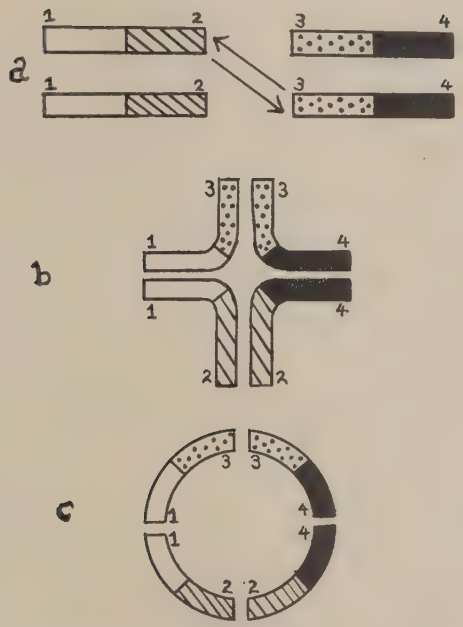

DIAGRAM 6

(a) Interchange between non-homologous chromosomes.
(b) Synapsis between interchanged and normal chromosomes.
(c) Resultant circle-formation.

tions which would be found when these complexes are combined in the formation of hybrids. They set out, therefore, to make and test such predictions, in an effort to test the correctness of the segmental interchange hypothesis.

To show how these predictions can be made, a single example will be presented. Suppose we take the case of *rigens* · *ʰJohansen* (*rigens* is the egg complex of *muricata,* *ʰJohansen* is the single type of genom produced by the homozygous California race, known at present as *"Johansen"*). The configuration of this hybrid was successfully predicted some years ago (25). The argument employed at that time was long and involved, owing to the fact that it was not then known that *acuens* gives ⊙ 4 and 5 pairs with *flavens* (27). Knowing this fact, a much simpler line of reasoning

is sufficient to show that *rigens* · *hJohansen* must have ⊙ 8 and 3 pairs; and since this simpler reasoning will illustrate the method as well as the more complex reasoning originally used, it will be employed.

We start with a knowledge of the segmental arrangements of certain complexes as a background; formulae for these complexes are as follows (each chromosome is represented by 2 digits joined by a dot, each digit representing an end segment):

hookeri has	1·2	3·4	5·6	7·8	9·10	11·12	13·14
flavens "	1·4	3·2	5·6	7·8	9·10	11·12	13·14
velans "	1·2	3·4	5·8	7·6	9·10	11·12	13·14
hJohansen "	1·2	3·4	5·6	7·10	9·8	11·12	13·14
acuens "	1·4	3·2	5·6	7·10	9·8	11·12	13·14

Rigens gives ⊙ 6 and 4 pairs with *hookeri;* ⊙ 4, ⊙ 6, 2 pairs with *flavens;* ⊙ 8, 3 pairs with *velans;* and ⊙ 4, ⊙ 8 and 1 pair with *acuens.* Its configuration with *hJohansen* is the subject of prediction.

Since *rigens* gives 4 pairs with *hookeri* and only 2 pairs with *flavens,* it has 2 more chromosomes with ends arranged as in *hookeri* than it has chromosomes whose ends are arranged as in *flavens.* But there are only 2 *hookeri* chromosomes which are not like those in *flavens;* consequently, these must be the ones which *hookeri* has in common with *rigens,* but which *flavens* does not have. *Rigens,* therefore, has 1·2 3·4. The fact that it has 1·2 3·4 will account for the presence of ⊙ 4 in *rigens* · *acuens.* But *rigens* gives with *acuens* not only ⊙ 4, it gives also ⊙ 8, and the latter must involve 4 of the last 5 chromosomes, because the first two are involved in the ⊙ 4. Since the last 5 chromosomes, however, are the same in arrangement in *hJohansen* and *acuens,* any configuration which *rigens* gives with the last 5 chromosomes of *acuens,* it will also give with the last 5 chromosomes of *hJohansen*— in other words, it will give ⊙ 8 with *hJohansen* also. But both complexes having 1·2 3·4, the entire configuration in *rigens* · *hJohansen* must be ⊙ 8 and 3 pairs.

Several months after this prediction was arrived at, opportunity came for examining this hybrid, and it was found to have the predicted configuration. More than 40 such predictions have been made, tested and published by Emerson and Sturtevant (46, 48, 49, 97), Cleland and Blakeslee (4, 28, 29) and the writer alone

(24, 25), and the only one of these that was wrong was one which was based upon a mistake in data. When this mistake was detected it was found that the revised prediction which alteration in the data made necessary was correct (27). We may say, therefore, that there have been no failures in predicting the chromosome configurations of various hybrid combinations; there has been, on the other hand, 100% success.

This can mean but one thing, namely, that the premises upon which the arguments have been based which have resulted in these predictions are correct. The premises are: (1) that the gene complexes making up the various species have diverse arrangements of the end segments of their chromosomes; (2) that each complex has its own specific and characteristic arrangement which it retains indefinitely or until a successful segmental interchange alters it. But naturally the existence of so many different arrangements of ends can have come about in only one way, namely, through a process of segmental interchange. The only other alternative is to give up the evolutionary point of view entirely in accounting for the origin of the various oenotheras and their complexes. We may say, therefore, that segmental interchange has been shown to be not only a possible explanation for circle formation in *Oenothera,* but the *only* possible explanation compatible with an evolutionary point of view.

Let us now return for a moment to the question as to whether the union of chromosomes end to end is responsible for the extensive linkage of genes so characteristic of this genus. According to the segmental interchange concept, the force which holds the chromosomes together in a circle is the same as that which holds paired chromosomes together, namely, the force exhibited during synapsis, whatever that force may be. But chromosomes which synapse even in part are always, in a diploid, the one of paternal, the other of maternal origin. Since, therefore, the force which holds together adjacent chromosomes in a circle is synapsis, it must necessarily follow that these are alternately of paternal and maternal origin. This necessary and unavoidable conclusion places our former assumption upon an unassailable foundation. Each chromosome occupies a definitive position within the circle, next to the two chromosomes which carry end-segments homologous with its own. If a given chromosome is paternal in derivation,

the two with segments homologous with its own must be maternal, and *vice versa.* Paternal and maternal chromosomes, therefore, alternate and the separation of adjacent chromosomes to opposite poles, which is an observed fact, must necessarily result in the separation of all paternal chromosomes and genes in the circle to one pole, and all maternal chromosomes and genes to the other. This accounts for complex-formation and for the apparent linkage of all genes in these chromosomes into a single linkage group as long as the chromosomes remain attached. It may be asserted, therefore, that the connection between chromosome concatenation and extensive genetical linkage in *Oenothera* is completely established.

PHYLOGENETIC IMPLICATIONS OF CYTO-GENETIC FINDINGS

A large number of species and hybrids of *Oenothera* have now been studied cytologically. From the standpoint of phylogeny, it is valuable, first of all to inquire into the frequency with which the various configurations have appeared during these studies.

Species. A total of 54 distinct races belonging to the sub-genus *Onagra* (which includes all the forms so far studied genetically) have been examined cytologically. These races fall into two very distinct groups from the standpoint of chromosome configuration: 35 of them show large circles (29 have ⊙ 14, 4 have ⊙ 12 and 2 have ⊙ 6, ⊙ 8); and 19 show small circles or none (10 have 7 pairs, 9 gave a variety of configurations in the first garden-grown generation, most individuals having 7 pairs or ⊙ 4, a few 2 ⊙s 4 or ⊙6). Seven of the 15 possible chromosome configurations have been conspicuous by their absence in natural races. This segregation into 2 clearly distinct groups (one with large circles and one with small circles or none) is extremely suggestive and will be referred to again later.

Hybrids. The chromosome configurations of 282 different hybrid combinations have been determined, this figure including only those hybrids whose parents have both been natural races, as opposed to mutants in pedigreed lines. Every one of the 15 chromosome configurations possible in diploids has been obtained within this assemblage, as will be seen from the solid line curve in chart 1. The hybrids so far studied, therefore, run the entire gamut from those with no pairs to those with nothing but pairs;

a fair majority of forms having fewer than 3 pairs, but a goodly proportion having from 3 to 7 pairs.

What, if any, phylogenetic significance can be found in these facts? Obviously, the presence of a large circle in a plant means that the sets of chromosomes uniting to produce this plant are quite dissimilar from the standpoint of segmental arrangement; on the other hand, the presence of mostly or entirely paired chromosomes is indication of the presence in the plant of chromosome sets whose segmental arrangements are similar or identical. We may now ask ourselves whether similarity in segmental arrangement is significant from the standpoint of phylogeny. Similarity in external morphological characters suggests, in many cases, phylogenetic affinity; does similarity in segmental arrangement have a similar significance?

Similarity in segmental arrangement may or may not have phylogenetic significance, depending upon whether segmental interchanges can occur between any two ends with more or less equal facility or whether they are restricted. If they are to some extent restricted, if some exchanges can occur much more easily than others, if many exchanges are perhaps impossible, it is to be expected that similarities in segmental arrangement will often be found among the complexes present in nature and that, therefore, little or no phylogenetic importance can be attached to such similarity. To take an extreme example: suppose that interchanges can occur only between chromosomes 1·2 and 3·4; then we can have, in addition to the original segmental arrangement (1·2 3·4 5·6 7·8 9·10 11·12 13·14), only 1·4 3·2 and (or) 1·3 4·2, the other chromosomes being alike in all complexes. Any cross we might make, in this event, no matter what the species used in the cross, would yield mostly paired chromosomes and would bring together genoms with similar segmental arrangements. This, of course, is an extreme example, but it serves to bring out the point that narrow restriction in the number of possible interchanges must result in reduction in the number of possible segmental arrangements, and hence must increase the chances of paired chromosomes being found when the various complexes are brought into combination. Similarity in segmental arrangement would not in this event necessarily mean close phylogenetic affinity.

On the other hand, it may be that interchanges can occur at ran-

dom, *i.e.*, with more or less equal facility between any two ends. If this is true, we would hardly expect to find much in the way of similarity between the various complexes existing in nature. This will be clear from a study of table 1 in the 1932 paper of Dr. Blakeslee and the writer (chart 1).

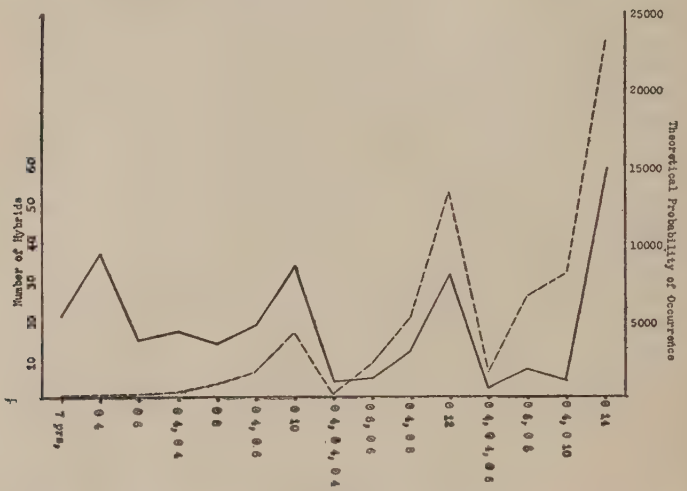

CHART 1

Chromosome Configurations Shown by Interspecific Hybrids of Onagra
Solid line shows number of hybrids with each configuration. (Scale on left)
Broken line shows theoretical probability of occurrence of each configuration, assuming that all ends of chromosomes can interchange with more or less equal facility. (Scale on right)

If we shuffle the ends of 2 sets or complexes of 7 chromosomes, arrange the ends of each set at random and then unite the 2 sets with like ends synapsing, we will find that the chances of a large number of pairs being found, *i.e.*, of great similarity in the arrangement of ends in the associated genoms, are very small indeed, whereas the chances of a large circle being formed are relatively very great. Thus, in the table, we find that there are 23,040 times as many combinations which will produce ⊙ 14 as combinations which will produce 7 pairs; there is but one chance for the production of 7 pairs to 23,040 chances for the production of ⊙ 14. Notice also that the chances for formation of large circles in general

are relatively great (in contrast to 1 chance for the production of 7 pairs, there are 13,440 chances for ⊙ 12, 4,032 chances for ⊙ 10); on the other hand, the chances for production of many pairs are small (but 21 chances for 5 pairs and 140 chances for 4 pairs in comparison with 23,040 chances for ⊙ 14). Obviously, if interchanges occur with any approach to randomness in nature, the chances of finding complexes with similar segmental arrangements (complexes which will give many pairs with each other) will be relatively slight.

This being the case, similarity in segmental arrangement, unless found with extreme rarity, will have to be, in general, ascribed to phylogenetic affinity. Complexes are similar in segmental arrangement because they have had a relatively recent common origin from which they have not as yet had time to deviate materially.

Now, similarities in segmental arrangement are not found with extreme rarity but have been observed rather frequently. Does this mean that interchanges are restricted as to the ends between which they can occur, or is it true that interchanges can occur between any two ends, and that similarities in arrangement are due to a close relationship between the complexes which show them?

All of the evidence so far accumulated seems to indicate that complexes with similar segmental arrangements are indeed closely related in most cases and that, therefore, similarity in segmental arrangement is on the whole indicative of close relationship. This evidence will now be briefly reviewed.

(1) *Oenothera* races which are highly heterozygous have large circles, whereas those which are mostly or entirely homozygous have mostly or entirely paired chromosomes. Thus, all of the 35 races with large circles which have been analyzed sufficiently for a statement to be made, have been found to be complex-heterozygotes, their genoms being dissimilar; on the other hand, the 19 races with small circles or none have turned out to have genoms which are identical, or at least similar to each other. Races which are intermediate in chromosome configuration have been conspicuous by their absence. This all seems to suggest that the presence of mostly paired chromosomes in a wild race is an indication of close relationship between the genoms making up this species.

(2) Occasionally, one of the complexes of *lamarckiana* ex-

changes a portion of itself for a corresponding portion of the opposing complex, giving up its lethal but receiving no lethal in return from the other complex. The modified alethal complex thus formed is then capable of existence in double dose. Consequently, a mutant arises in which this complex is present twice. Naturally, such a mutant is completely homozygous. It is interesting to note that all such mutants (there are 4 known at present) have *7 pairs* of chromosomes. Then there are species in which one complex is alethal or easily rendered alethal, *e.g., acuens* of *grandiflora* or *flavens* of *suaveolens.* Individuals are occasionally produced by these species and survive, in which these complexes are present twice. Such individuals are again naturally quite homozygous, except perhaps for the lethal, and they also have wholly paired chromosomes. In all these cases, the presence of wholly paired chromosomes, *i.e.,* complete identity in segmental arrangement, goes along with practical or absolute identity in genetic composition.

(3) A third line of evidence is derived from a comparative study of complexes in different species. Renner has for many years been studying various complexes genetically, analyzing them from the standpoint of their gene content. He has found certain cases in which complexes, which are in different species, are, nevertheless, more closely related to each other than they are to the complexes which are their normal associates (59, 80).

In the meantime, the writer has been studying many of these same complexes from the standpoint of the chromosome configurations which they give when brought into combination with each other, and has found that the complexes which Renner considers to be closely related give mostly pairs when united and, therefore, have similar segmental arrangements, whereas the complexes which he regards as quite unrelated give large circles with each other (23). Here again is clear-cut evidence that the presence of numerous pairs in a complex-combination is evidence of close relationship between the complexes involved.

All of these lines of evidence, therefore, seem to indicate that the number of pairs present in a form is a rough indication of the degree of relationship existing between the complexes making up this form, which means that chromosome configuration becomes an important tool for the study of *Oenothera* phylogeny, affording, as

it seems to do, a simple and rapid method of determining close relationships between complexes. Such a tool should be a very useful supplement to taxonomic and other approaches to the problems of species relationship.

With a view to testing the usefulness of this tool, therefore, and utilizing it, so far as possible, in the solution of problems of *Oenothera* phylogeny, Prof. P. A. Munz of Pomona College and the writer have begun a systematic survey of the oenotheras belonging to the sub-genus *Onagra* (the subgenus which has furnished almost the sole material for cyto-genetic studies so far). The various wild races are being considered from both the systematic and the cyto-genetic points of view. By comparing the conclusions reached by these alternative approaches, it is hoped that a degree of insight into species relationships and the forces which operate in species formation will be attained, such as has been rarely found possible. These joint studies are still in an incipient stage, but a preliminary cyto-genetic study of races from California by the writer (26) has led to the following conclusions based upon cyto-genetic data, some of which, at least, have been found to agree with conclusions based upon systematic studies:[1]

(1) The onagras of California are characterized by the presence of mostly or entirely paired chromosomes, in contrast to those from other regions, almost all of which, so far studied, have shown large circles.

(2) California races have shown no evidence of lethal factors, which are so preeminently characteristic of races from elsewhere.

(3) California races have very little seed and pollen sterility; other onagras show, as a rule, high sterility.

(4) The gene complexes or genoms composing plants of the California races are identical or at least similar, genetically; those found in other races are usually very distinct. Consequently, races from outside California may ordinarily be referred to as "complex-heterozygotes"—those from California cannot be so designated.

(5) The various races in California and the vicinity of California resemble one another closely in the arrangement of end segments, as is shown by the fact that hybrids between them yield entirely or mostly paired chromosomes. Assuming that similarity

[1] The writer acknowledges with gratitude generous support from the Penrose Fund of the American Philosophical Society, which has made this study possible.

in segmental arrangement is an indication of near relationship, this fact indicates that these races are phylogenetically close to each other.

(6) Races of the California group, when crossed with races from other regions, produce hybrids with a great variety of chromosome configurations—almost all 15 of the possible chromosome arrangements that can be found in 14-chromosome individuals having been observed in these hybrids.

Most of the complexes from outside California show no very close relationship to the California group, producing large or relatively large circles with them. But curiously enough, some complexes belonging to extra-California races have proved to be essentially of the California type, having segmental arrangements of the same general type and thus giving mostly paired chromosomes when combined with California genoms. The complexes which have shown this close relationship are *velans, flavens, acuens, excellens* and, to a slightly lesser degree, *rigens* and *fascians.* It is interesting that this list includes the two complexes, belonging to complex-heterozygotes, which are known to be alethal or, at the most, possessed of a semi-lethal, namely, *acuens* and *flavens.* It is also interesting that *velans, flavens* and *acuens* are complexes which Renner had decided earlier were closely related genetically to each other and to *hookeri,* one of the California genoms.

The races which contain these 6 complexes were obtained from widely scattered regions, although there is a possibility that they were not in all cases indigenous in the regions where they were found—in fact, in the case of 2 of them, which belonged to races picked up in Europe, it is evident that they were not in their native haunts. The races picked up in America came from such widely separated regions as Illinois, Alabama and Massachusetts, a long distance, therefore, from California.

We find, therefore, that races from regions far removed from California, so far as they have been studied, have complexes which for the most part show little or no resemblance in segmental arrangement to those from California, but in some cases they possess one complex of the California type, associated with a complex which is not of the California type.

These facts raise a number of interesting questions: (1) How are we to account for the very different cyto-genetic behavior of

onagras from California and those from other regions? (2) To what extent are complexes of the California type actually distributed in the various areas outside California and how is it that they should have found their way into these regions? (3) How are the complexes which are not of the California type to be classified from the standpoint of segmental arrangement? Do they also belong to a single type, or do they fall into several categories, or are they so varied as to escape classification? (4) Is there any evidence that segmental arrangements not of the California type are as characteristic of certain other geographical areas as the California type is characteristic of the far west—in other words, is there evidence with regard to the probable origins of complexes not of the California type?

Complete answers to these and other questions must be sought through further investigation; at present the best one can do is to hold tentative opinions or working hypotheses. They indicate, however, some of the directions in which present research is moving and the sort of problems which it may be possible to solve through a combined taxonomic and cyto-genetic investigation of the genus.

TELOSYNAPSIS OR PARASYNAPSIS?

Oenothera has been considered, since the beginning of Gates' and Davis' cytological studies on the genus (39–41, 50–52), an outstanding example of the phenomenon of telosynapsis. In recent years, the position that *Oenothera* is telosynaptic has been attacked with vigor and it is now apparent that the original position requires modification.

The essential differences between the telosynaptic and parasynaptic interpretations of meiotic prophase behavior are these:

(1) According to telosynaptists, the side by side association of homologous chromosomes does not occur until late prophase ("strepsinema," "second contraction"); hence, the spireme previous to this stage (in a stage corresponding to "pachyphase") is univalent. According to the parasynaptists, synapsis occurs early in prophase and, as a result, the pachyphase spireme is bivalent.

(2) The early spireme, on the telosynaptic interpretation, is continuous and the chromosomes are united end to end until the time for side by side pairing arrives. This end to end union does

not imply homology between the attached ends. According to the parasynaptic interpretation, however, there is no continuous spireme. The chromosomes are not attached end to end, their only association in prophase being that of synapsis, which type of union does definitely imply homology between the associating parts.

All the earlier workers in *Oenothera* cytology adopted the telosynaptic point of view, chiefly for 2 reasons: (1) Splits were not visible in the stages which would correspond to pachyphase and early diplophase in other organisms; the spireme was thus thought to be univalent. (2) The early spireme appeared to be continuous and the chromosomes in diakinesis were mostly attached end to end to form a univalent chain. The workers adopting the telosynaptic point of view included Gates (50–52), Davis (39–41), Cleland (13–15, 17, 18), Håkansson (55, 56), Sheffield (89, 90), Illick (61, 62), Kulkarni (64, 65), Hedayetullah (58), Capinpin (8, 9), Sinotô (96).

On the other hand, certain writers have expressed skepticism of the telosynaptic point of view as applied to *Oenothera,* some of these supporting without qualification the parasynaptic interpretation. These authors included Schwemmle (88), Kihara (63), Boedijn (6, 7), Leliveld (67, 68), Darlington (35, 37, 38), Catcheside (10–12), Emerson (45), Gates and Goodwin (53), Wisniewska (108), Weier (107).

There is not space in the present article to enter into the pros and cons of this subject. Suffice it to say that the bulk of evidence seems now to support the conclusion that the chromosomes in *Oenothera* are associated side by side at least at the ends. How far back from the ends synapsis occurs is at present a matter of controversy. Darlington, strongest proponent of parasynapsis in *Oenothera,* argues for the existence of "differential segments" in the centers of the chromosomes, in which the genetic material is not necessarily arranged in the same order in the various complexes. This would effectively prevent synapsis in the interstitial segments, except in rare instances where homologous bits were so situated in opposing complexes that they could synapse. If Darlington's concept is correct, as it may well be, at least in some degree, it naturally follows that most of each chromosome in "pachyphase" is unpaired and that, therefore, a considerable portion of the thread system at this stage is univalent. A really criti-

cal piece of work has yet to be done to settle the question as to how much of the chromosome is paired and how much unpaired at this time. At present, however, we may accept the concept that chromosomes in *Oenothera* synapse at the ends, leaving the question open as to whether the central regions follow suit. If synapsis is found to be general throughout the length of the chromosome, *Oenothera* will fall into line with most other organisms in constituting a more or less typical example of parasynapsis. If, however, Darlington's contention is correct, *Oenothera* will have to be considered somewhat exceptional, for, on the one hand, it will be seen to be parasynaptic at the ends, but, on the other hand, will show for the most part the univalent spireme claimed by telosynaptists as typical of the meiotic prophase stages.

The aspects of the cyto-genetic investigation of *Oenothera* which have been reviewed in the present paper are some of those with which the reviewer is in most intimate touch. There are other important aspects which have had to be omitted for lack of space. Thus, there are the interesting embryological studies of Renner and his students; the significant contributions of Gates, Davis, Håkansson and others to our knowledge of non-disjunction, trisomy, haploidy and polyploidy in the group; as well as investigations into the nature of *Oenothera* "mutants," in general, and into the effects of environmental conditions and of radiations upon cytological and genetical behavior. A review of these aspects of the subject must await a more extended survey of *Oenothera* research.

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Glossary

crossing-over: an exchange of parts between homologous chromosomes. It results in the separation of genes which are ordinarily linked.

diakinesis: the last stage in the prophase of the first meiotic division.

diplophase: the stage in prophase of the first meiotic division when the synapsed chromosomes begin to fall apart.

fragmentation: breakage in a chromosome.

genom: the entire set of chromosomes inherited from one parent.

independent segregation: the segregation of maternal and paternal chromosomes of any homologous pair independently of the direction of segregation in any other pair.

lethal (factors): factors which render inviable an organism possessing them in a homozygous condition, or (occasionally in plants) factors which prevent the functioning of gametes.

lethals, balanced: lethal factors in opposite genoms. Individuals possessing them appear to breed true because one half of the progeny are homozygous for a lethal and perish, or because one genom fails to function as sperm, the other as egg.

linkage: the association of genes in the same chromosome, and the consequent tendency of the characters which they govern to be inherited together.

meiosis: the period during which homologous chromosomes, or parts of chromosomes, pair (synapse) and then separate, so that the number of chromosomes is reduced from the diploid ($2n$) to the haploid (n). It immediately precedes spore or gamete formation, and consists of two successive nuclear divisions.

non-disjunction: the failure of homologous chromosomes to separate into different daughter nuclei at meiosis.

pachyphase: the stage of the first meiotic prophase during which the chromosomes are closely synapsed.

polyploidy: the condition when more than two sets of homologous chromosomes are present in an individual.

chromosome rings or circles: chromosomes attached end to end in a ring. Such formations occur at meiosis in many oenotheras and in certain other forms.

segmental interchange: an exchange of segments between non-homologous chromosomes.

spireme: during early stages of nuclear division, the chromosome is in the form of a thin thread or spireme.

synapsis: pairing of homologous chromosomes during meiosis.

translocation: transfer of material from one region of chromosome to another region of the same or different chromosome. Reciprocal translocation, or mutual exchange of material, is segmental interchange.

trisomy: the condition in which one of the chromosomes is present in triplicate.